Wastewater use in urban agriculture: an exposure and risk assessment in Accra, Ghana

Prince Antwi-Agyei

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Supervisor: Dr Jeroen Ensink

Department of Disease Control

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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Research group affiliation: Environmental Health Group, LSHTM

Declaration

I, Prince Antwi-Agyei, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed Date

FULL NAME: PRINCE ANTWI-AGYEI

Abstract

In order to minimize the health risks to agricultural workers, and consumers of wastewater irrigated produce, the World Health Organisation has developed guidelines for the safe use of wastewater in agriculture. This study sought to test the appropriateness of the current Quantitative Microbial Risk Assessment model and the multiple-barrier approach advocated by the WHO guidelines.

Over a one year period, over 500 produce and ready-to-eat salad samples were collected from fields, markets, and kitchens in Accra, Ghana, and over 300 soil and irrigation water samples were collected. All samples were analysed for *E. coli*, human adenovirus and norovirus using standard microbiological procedures. In addition, almost 700 participants including farmers, food vendors, and consumers were interviewed and observed to assess critical exposures associated with the transmission of faecal pathogens.

The results showed that irrigation water was significantly more contaminated than farm soil, though exposure to soil was found to pose the key risk to farmers due to hand-to-mouth events. Over 80% of produce samples were found contaminated with *E. coli*, with street food salad being the most contaminated (4.23 Log *E. coli*/g), and that consumption of salads did not meet health standards. Risk factors identified for produce contamination included farm soil contamination, wastewater use for irrigation, poor hygiene, and operating with a hygiene permit. Awareness of the source of irrigation water was low, but despite the high awareness of health risk, consumers did not prioritize health indicators when buying produce from vendors. Similarly, farmers' awareness of health risk did not influence their adoption of safer farm practices. The study recommends the promotion of interventions that would result in more direct benefits to producers and vendors, together with hygiene education and inspection, hygiene certification and enforcement of food safety byelaws in order to increase the uptake of the multi-barrier approach.

PhD related documents

2014: Antwi-Agyei, P., Cairncross, S., Peasey, A., Bruce, J., Baker, K., Ensink, J.H.J. (submitted). A risk assessment for the use of wastewater in agriculture in Accra, Ghana. SNOWS (Scientist Networked for Water and Sanitation) consortium.

2015: Antwi-Agyei, P and J., Ensink, J.H.J. (Published). Video documentary on Wastewater irrigation in urban agriculture in Accra, Ghana. SHARE (Sanitation and Hygiene Applied Research for Equity) project, http: http://www.shareresearch.org/NewsAndEvents/Detail/Using-Wastewater-in-Urban-Agriculture

2015: Prince Antwi-Agyei, Sandy Cairncross, Anne Peasey, Vivien Price, Jane Bruce, Kelly Baker, Christine Moe, Joseph Ampofo, George Armah, Jeroen H. J. Ensink (Submitted). A farm to fork risk assessment for the use of wastewater in agriculture in Accra, Ghana. PLOS ONE

2015: Prince Antwi-Agyei, Anne Peasey, Adam Biran, Jane Bruce, Jeroen H. J. Ensink (Submitted). Risk perceptions of wastewater use for urban agriculture in Accra, Ghana. PLOS ONE

2015: Prince Antwi-Agyei, Adam Biran, Anne Peasey, Jane Bruce, and Jeroen H. J. Ensink (Submitted). A faecal exposure assessment of wastewater farm workers in Accra, Ghana. Water Research.

Dedication

This PhD work is dedicated to my parents, Miss Comfort Adi-Asah, and Mr Samuel Jonathan Antwi-Agyei, both of blessed memory. I thank both of you for instilling the value of good education in my life. Rest in Peace.

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List of abbreviations

AIC	Akaike information criterion
AF	Attributable Fraction
AMA	Accra Metropolitan Assembly
β-Poisson	Beta Poisson
CFU	Colony Forming Unit
^{O}C	Degree Celsius
CLTS	Community Led Total Sanitation
CI	Confidence Interval
d	day
DALY	Disability Life Adjusted Years
DFID	Department for International Development
DNA	Deoxyribonucleic acid
dS	deciSiemens
E. coli	*Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EHG	Environmental Health Group
EHSD	Environmental Health and Sanitation Directorate, Ghana
FAO	Food and Agriculture Organisation
FDA	Food and Drugs Authority, Ghana
FGD	Focus group discussion
g	gram
GAMA	Greater Accra Metropolitan Area
GSS	Ghana Statistical Service
h	hour
ha	Hectare
HACCP	Hazard Analysis and Critical Control Points
ICMSF	International Commission on Microbiological Specification of Foods
HAV	Human adenovirus
IC RT-PCR	Immunocapture reverse transcription polymerase chain reaction
IQR	Inter quartile range
IWMI	International Water Management Institute
l	litre
LLOD	lower limit of detection
LMICs	Low and middle income countries
LSHTM	London School of Hygiene and Tropical Medicine
m	month
MC	Monte Carlo
MEST	Ministry of Science and Technology, Ghana
MLGRD	Ministry of Local Government and Rural Development, Ghana
MOFA	Ministry of Food and Agriculture, Ghana
MPN	Most probable number
NGO	Non-governmental organisation
NV-GI	Norovirus genogroup I
NV-GII	Norovirus genogroup II
NY	New York
OR	Odds ratio
PCR	Polymerase Chain Reaction
PBS	Phosphate buffered saline

RNA	Ribonucleic acid
pppd	per person per day
pppy	per person per year
QMRA	Quantitative Microbial Risk Assessment
RT-PCR	Real Time Quantitative Polymerase Chain Reactions
SANIPATH	Sanitation Pathways
SHARE	Sanitation and Hygiene Applied Research for Equity
SNOWS	Scientist Networked for Outcomes in Water and Sanitation
TR	Tolerable risk
TTC	Total thermotolerant coliform
UCL	University College London, UK
UN	United Nations
US	United States
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet light
WHO	World Health Organisation
y	year

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Chapter 1: Wastewater use and Perspectives

Photo 1.1: A farmer in Accra, Ghana, manually irrigating lettuce with wastewater

1.1 Urbanisation, water scarcity and wastewater use in agriculture

The world population is estimated to reach over 9.5 billion by 2050, with 66% of the population expected to live in cities [1]. Rapid population growth and urbanization inadvertently mean an increased generation and production of faecal sludge and wastewater within cities. The use of wastewater[1] in (urban) agriculture has become common in many countries as a result of rapid urbanisation, the high costs associated with it, and a lack of wastewater treatment facilities and water scarcity [2]. Unlike the use of human excreta in agriculture which has been practiced for centuries in China and South-East Asia, the use of wastewater in agriculture is a more recent practice, particularly in Africa [3]. The practice is more common in arid and semi-arid countries where fresh water is scarce, and planners are forced to consider marginal quality sources as alternatives [4].

The use of wastewater can be a planned, and therefore regulated practice, though in many countries wastewater use is often unplanned and unregulated. Especially in low or middle income countries wastewater is often used untreated and unregulated [5]. Other countries like Jordan and Saudi Arabia have national policies that mandate the use of treated wastewater for irrigation [6], while Peru allows wastewater to be used for crops that are eaten cooked [7], and in Mexico, only treated wastewater that meets the World Health Organisation (WHO) guidelines are permitted for irrigating salad crops [7]. Wastewater use is also often referred to as either direct use (i.e. wastewater is taken directly from sewerage system, or wastewater treatment plants, without any dilution), or indirect use (i.e. wastewater is first disposed into a fresh water source or other water bodies before it is used by farmers). There is an issue with the definition of wastewater as it is unclear at what stage wastewater ceases to be wastewater especially in the case of disposal of wastewater into surface water bodies, and therefore with the definition of indirect wastewater use in agriculture. It is estimated that wastewater agriculture is practiced in 80% of all cities in low and middle income countries (LMICs), and that 24 million ha of agricultural land, (11% of all irrigated croplands) are irrigated with either raw or partially treated wastewater, though the exact extend of wastewater use in agriculture is unknown due to lack of data, and the diverse definitions of wastewater [8, 9]. Countries with large areas of irrigated land include China, Mexico and India (Figure 1.1).

[1] *Wastewater as used in this document covers domestic effluent of blackwater and greywater, water from commercial establishments and institutions and storm water and other run-off.*

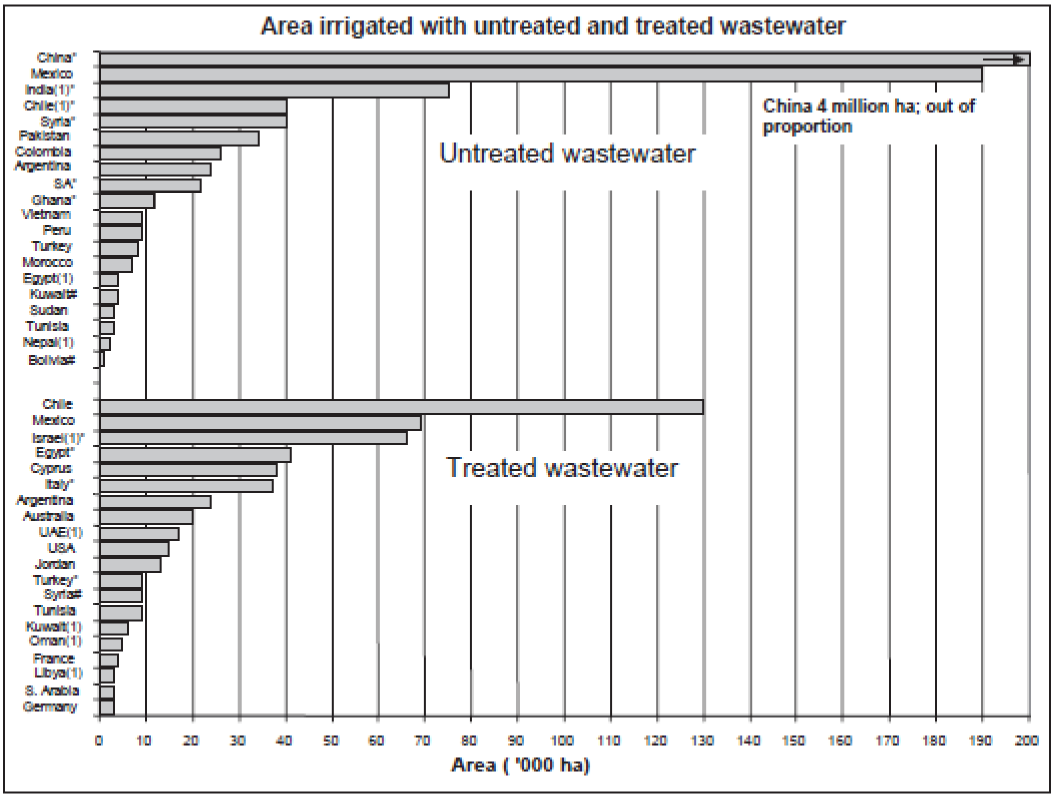


Figure 1.1 : Countries with greatest irrigated areas of treated and untreated wastewater.
Sources: Jimenez and Asano (2008), modified, for China: Xianjun *et. al* (2003)

The crops most commonly grown by farmers using wastewater are fodder and vegetables [3]. Vegetables in particular are popular with farmers as they satisfy the high market demand, and provide profit and livelihood to farmers [10, 11]. They are also more appropriate for the land-tenure system in most low and middle income countries where wastewater irrigated lands are often not owned by farmers, and as such farmers prefer to cultivate crops with shorter maturity periods [10].

1.2 Wastewater use in agriculture: benefits and risks

The use of wastewater in agriculture has the potential to raise both agricultural productivity, and the living standards of the poor [6]. Farmers often cite economic benefits as a major reason for wastewater use, as the wastes contain valuable nutrients needed for plant growth [4, 12]. For example, an estimated 1,000 m^3 of municipal wastewater used to irrigate one ha of agricultural land can contribute up to 62 kg of nitrogen, 69 kg of potassium, and 24 kg of phosphorous to soil nutrient; thus reducing the demand and use of chemical fertilisers [13]. Farmers also consider wastewater a more reliable source with all year round access [11, 14].

The use of wastewater also allows farmers to cultivate different types of crops, as wastewater availability will allow for more frequent irrigation, allowing the cultivation of crops with shallower rooting depths, which might result in higher cropping intensity and output [15]. In Pakistan and Mexico, studies have shown that wastewater farmers make cost savings due to the reduced use of chemical fertilisers compared to regular farmers [14]. For example, farmers in Mexico who irrigate about 140 ha of land annually make a cost saving of $135 per hectare per year, a substantial amount of money which would otherwise be used to purchase chemical fertilisers [16]. The proper use of wastewater can also help in recharging aquifers through infiltration, or in reducing surface water pollution as the wastewater gets 'treated' in the vadose zone of soil before reaching water bodies [6, 17]. In terms of financial gains, work in Pakistan has shown that farmers with access to wastewater (in this case untreated wastewater) could harvest more crops per year, and earned over US$ 600 per hectare per year more than farmers using regular irrigation water as a result of higher cropping intensities and savings in fertilizer costs [14, 18]. Similarly, higher crop yields (42% increase) and hence higher incomes arising from wastewater use in agriculture have been reported among farmers in Senegal and India [19, 20]. Higher crop yields also means improved food availability, and lower cost price of food, and hence ability of poor households to meet their nutritional needs and other health promoting activities [3].

Besides these benefits, the use of untreated, or partially treated wastewater in agriculture holds clear risks to both the environment and human health. These risks originate from pathogens and chemicals dissolved in wastewater. Although exposure to chemicals in wastewater (including heavy metals) could pose health risks to humans, evidence on the direct impacts is limited. Generally, the concentrations of most chemicals in urban wastewater (with limited inflow of industrial wastewater) are too low and tend not to result in acute health effects, or may require long-term exposure to have significant health impact [21, 22]. From a public health perspective, pathogens are therefore considered the primary risk especially with the use of untreated and partially treated domestic wastewater. The type and concentration of pathogens in wastewater will vary depending on the local conditions such as climate, season and on the background levels of infection found among the population [21]. In terms of disease transmission, Shuval *et al.*[23], built upon the "environmental classification of excreted infection" by Feachem *et al.*, [24], and used epidemiological considerations like: a) persistence in the environment, b) latency, c) infective dose and d) immunity, in order to rank the different pathogens found in wastewater and their associated health risk; thereby assigning the greatest health risk to helminths, followed by protozoa, bacteria and viruses.

In terms of persistence, pathogen survival depends on the type and conditions of the environment it is exposed to (Table 1.1). For example, in water pathogen survival is influenced by temperature, pH, and the levels of organic pollution, while on crops pathogen survival is determined by the type of crop, temperature, exposure to UV, and air humidity. In soils survival times depend on soil moisture content, levels of shade, levels of salt concentrations and soil type [22, 23]. Overall, pathogen survival times are shorter on crops compared to other environments [23].

Table 1.1: Survival of pathogens on crops surfaces, in soil and in water at 20 - 30^{o}C

Hazard	Survival on crops (days)	Survival in soil (days)	Survival in water (days)	Median ingestion dose
Bacteria	< 30 but usually < 15	< 70 but usually < 20	< 60 but usually < 30	Medium - High
Viruses	< 60 but usually < 15	< 100 but usually < 20	< 120 but usually < 50	Low
Protozoans	< 10 but usually < 15	< 150 but usually < 10	< 30 but usually < 15	Low/medium
Helminths	Many months	Many months	Many months	Low

Note: Low ($\leq 20^2$); Medium ($\approx 10^4$); High ($\geq 10^6$)
Adapted from [21, 23]

1.3 Wastewater use guidelines

In order to protect farmers working with wastewater, and consumers of wastewater irrigated produce from adverse health impacts; water quality guidelines and standards were developed. In the United States, the State of California where wastewater has been used since the 1890s was the first to develop regulations on the safe use of reclaimed water [25]. The first standard adopted by the California State Board of Health was in 1918, but a more comprehensive regulation directed mainly at the control of disease agents was enacted in 1968 [25]. The regulations have since undergone revisions with the current standards recommending the use of water of quality not exceeding total coliforms of 2.2 cfu/100 ml for irrigating crops that can be consumed uncooked [25].

In response to the strict water quality standards set in the USA, the WHO developed its first guideline in 1973, building upon the water reuse standards of the State of California in 1968. The guideline relied on water quality levels which could be achieved based on available treatment technologies at the time [26]. The guideline target was set at 100 faecal coliforms per 100 ml for unrestricted irrigation (edible crops, including those eaten uncooked) [26]. However, this water quality standard was considered to be overly strict, and practically impossible to achieve in low and middle income countries. The guidelines were revised in 1989 taking into account microbiological, technological and epidemiological evidence of actual risks to public health, rather than potential hazards indicated by the survival of pathogens on crops, wastewater and in soil [27]. A new guideline for unrestricted irrigation was set at 1,000 faecal coliforms 100/ml, and an intestinal nematode guideline of ≤1 ovum/litre was introduced [27]. Unlike the 1973 edition, the 1989 guidelines recommended health protection measures, e.g. crop selection/restriction, wastewater application measures, human exposure control and hygiene promotion in addition to wastewater treatment. These guidelines were considered straightforward, but some argued that they were more favourable to countries with comprehensive wastewater treatment systems [28], while others questioned the level of acceptable water quality needed to avoid excess enteric diseases among exposed populations [29]. Proponents of the much more stringent United States Environmental Protection Agency (USEPA) guidelines also considered the WHO standards as too lenient by not considering other factors such as acquired immunity [29]. On the other hand, the California water standards, or the USEPA water quality guideline of no detectable faecal coliform per 100ml may also not be practically feasible to attain in many resource constrained countries where secondary treatment options to high-level disinfection methods required to achieve this level of water quality are either unavailable, or non-functional as a result of high operational and maintenance cost of the facilities. Besides, most water bodies used for irrigation purposes have *E. coli* or faecal coliforms concentrations in excess of zero/100 ml, as is the case with the Musi River in Pakistan [30].

In order to strengthen the WHO guidelines, a review of epidemiological studies since 1989 was conducted but the review found a lack of good quality studies, and thus inadequate evidence to support an epidemiological approach to risk assessment based on water quality standards [31]. The wastewater guidelines were again revised in 2006 and presented a new approach based on the Stockholm Framework, and comprehensive risk assessment and management approaches. The guidelines combine the use of an epidemiological approach, and a Quantitative Microbial Risk Assessment (QMRA) model approach to ensure public health safety.

1.4 Evidence of wastewater use and Health risks

A critical review of wastewater epidemiological studies from 1985 to 2001 found that most of the studies investigating the association between wastewater use in agriculture, and health impacts were inconclusive [31]. Most of the studies had a number of design and analytical flaws that included: failure to define exposure at an individual level (misclassification bias), no water quality specified, no report on significance test, no control for potential confounding factors, no evidence of causality, or were in general methodologically handicapped. Some of the studies also used, or relied on proxy indicators to measure infection, or disease outcomes, while others relied on self-reported diseases, or clinical records to conclude on associated risks as was reflected in some studies conducted in Israel and Mexico [32-34]. Even in other studies where human samples (e.g. stools, blood) were taken for the prevalence of pathogens, no direct observations were used to determine exposure time and frequency to risk factors, but rather the use of interviews which could be prone to information and recall bias and hence may produce spurious results [31]. For example, observational studies in Vietnam which relied on interviews produced contrasting results; in one study exposure to wastewater increased the risk of helminth infection while another found no significant association between wastewater exposure and helminth infection in wastewater farmers [35, 36]. Although stool samples from participants were examined for helminth prevalence in these studies, exposure was only measured at the household level but more importantly no direct observations of exposures were made, but rather the use of interviews which could be prone to various forms of bias. In effect, most past studies estimated relative risks, and not attributable risks, and also failed to quantify the excess risk arising from wastewater use.

In urban or peri-urban agriculture, the use of wastewater can only pose an *actual* risk to health if all of the following occur: a) an infective dose of an excreted pathogen reaches the field or pond, or the pathogen multiplies in the field or pond to form an infective dose; b) the infective dose reaches a human host; c) the host becomes infected; and d) the infection causes disease, or further transmission. The risk remains a *potential* risk if only (a), or (a) and (b), or (a), (b) and (c) occur, but not (d) [23, 37].

1.4.1 Farmers' occupational health risk

Most previous studies investigating the effect of direct contact of untreated, or treated wastewater on health outcomes in farmers, their families and in nearby populations, had design and methodological challenges that influenced the validity of their findings [34, 38-40]. In addition, most of the studies also reported only relative risks (odds ratios), which do not necessarily provide a measure, or estimate of the risk contributed (attributable risk or population attributable fraction) to the direct exposure of untreated wastewater. There are, however, few studies that have attempted to estimate the attributable risk arising from exposure to untreated and treated wastewater, or have controlled for potential confounding factors. For example, a study in Hyderabad, India, attributed hookworm infections among adult farmers to direct exposure of untreated wastewater with odds ratio (OR) of 3.5 after controlling for potential confounders [41]. Direct contact with polluted rivers and ponds used for irrigation was also associated with increased risk of diarrhoea diseases among adults in Vietnam (OR = 2.4, 95% confidence interval (CI) = 1.2 – 4.7, attributable fraction of population, AF = 27%, [42]). Studies in Mexico also linked the level of wastewater treatment (including the length of wastewater retention time in a series of reservoirs) to the risk of *Ascaris* infection among farmers [34, 43, 44].

A study in Vietnam established that wastewater contact was the principal risk factor (OR = 1.98, AF = 35%) to diarrhoea disease in adults engaged in wastewater [45]. In Pakistan, farmers who used untreated wastewater were estimated to have a threefold increase risk of asymptomatic *Giardia intestinalis* infection (attributable risk of 28%) compared to farmers using non-wastewater sources for irrigation [46]. In general, there is some evidence linking direct contact with untreated wastewater to increased risk of helminth infection (especially *Ascaris* infection) with the effect being more pronounced in children than in adults in situations where children are involved in agriculture [31].

1.4.2 Consumption health risk

Similar to farmers' occupational risks, there are few studies that have provided sufficient evidence on the risk of consuming wastewater irrigated produce. Most of the studies rely on the quality of produce linked to wastewater use as evidence of health risk without any evidence associating wastewater use to disease outbreaks or infections among consumers [47-51]. Most other studies report only on the relative risk, without necessarily presenting how much of the risk is actually attributed to the use of untreated or treated wastewater. Few studies, however, provided some level of evidence by overcoming some of the above limitations. A prospective cohort study in Mexico produced an adjusted OR for *Ascaris* infection of 3.9 (men, with consumption attributable risk of 14%) and 2.4 (children, consumption attributable risk of 25%) for consumption of irrigated vegetables by farming families after controlling for confounding variables [43]. In Santiago, Chile, a study showed that the consumption of uncooked vegetables irrigated with untreated wastewater was associated with significant increase risk in seroprevalence to *Helicobacter pylori* (Relative risk 3.3, $P < 0.001$) [52]. Blumenthal *et al*. [53] also reported that medium to high consumption of onions irrigated with water stored in a single reservoir (water quality of 10^4 thermotolerant coliforms) was associated with a twofold or greater risk of diarrhoea disease, and also a twofold increase in seroresponse to norovirus associated to the consumption of green tomatoes. Overall, there is evidence to suggest that the use of untreated, or partially treated wastewater on salad crops could result in an increased risk of helminth and bacterial infections as well as symptomatic diarrhoea disease among consumers, though it is unclear the extent of treatment needed to prevent this excess risk [31].

1.4.3 Water quality and health risk

Despite the evidence linking wastewater to health risk, it remains unclear on the threshold of microbial quality of irrigation water beyond which could result in adverse health effect to farmers and consumers. For example, the review by Blumenthal and Peasey [31] found that though there was significant risks of gastro-intestinal, and other infections to consumers of crops and farm workers, the level of water quality that could result in these risks depended on several factors including the method/type and extent of treatment, the method of irrigation applied, the people exposed (adults or children, nearby population), and climatic conditions (including seasonality). Other factors were the type of crop irrigated, adoption of effective hygiene measures, the type of pathogen, and the time between harvest and consumption. Moreover, most of the studies failed to report the quality of irrigation water, or wastewater used for irrigation. The review conducted by the WHO concluded that a relaxed guideline of at least $10^4 - 10^5$ faecal coliforms/100ml would be adequate to protect human health but concentrations of ≤ 1 ovum/l for nematode may not necessarily ensure health safety especially where surface irrigation (e.g. flood irrigation) is used, and children under 15 years are exposed to wastewater [31]. A more recent study conducted in India recommended a provisional guideline of 15 eggs for unrestricted irrigation if hookworm was the main risk [41]. However, the continuous discussion on the validity of the WHO water quality standard was one of the main reasons for the revision of the 1989 guidelines and the use of health-based targets and a QMRA approach in the 2006 guidelines.

1.5 QMRA and the new WHO guideline approach

The 2006 WHO guidelines are built around a health and an implementation component, both of which must be considered in order to achieve adequate health protection. For the health component, the guidelines define a health based-target (e.g. DALYs or absence of disease, wastewater quality, performance targets for specified treatment technology) and identify health protective measures to achieve this. It recommends the use of a Quantitative Microbial Risk Assessment (QMRA), and the application of the multi-barrier approach for managing health risks instead of relying solely on wastewater treatment to achieve water quality thresholds. One implication of the new guidelines is that governments from different countries can develop, and set their own local health-based targets, or standards based on prevailing local conditions such as social, cultural, environmental and economic conditions, as well as the particular epidemiological scenarios in the countries after health risks have been estimated [21, 31].

1.5.1 Quantitative Microbial Risk Assessment (QMRA)

QMRA is the application of risk assessment principles to estimate the consequences from a planned or actual exposure to infectious microorganisms [22]. The QMRA as used in the 2006 guidelines estimates potential excess risk of infection, or disease of specific pathogens to human health (with potentially much greater sensitivity) and may be used in conjunction with epidemiological evidence as well as studies of environmental behaviour of microbes [21, 54]. The use of QMRA models is also a much quicker way of estimating risk that would otherwise be more difficult and costly to measure with epidemiological studies [21].

The QMRA process or methodology for wastewater assessment uses the same conceptual framework for undertaking chemical risk assessment which includes hazard identification, hazard characterization (including dose-response), exposure assessment and risk characterisation [21, 22] (Box 1.1). In the WHO guidelines, the QMRA model uses its input data (e.g. pathogen concentrations, frequency and quantity of exposure to source of hazard, pathogen die-offs, disease/infection ratio etc.) and combines this with the 10,000-trial Monte Carlo risk simulations to estimate risk. The estimated risk is then used to determine pathogen reductions needed to achieve a disease burden of ≤10^{-6} DALY (disability-adjusted life years) per person per year (pppy). The 10^{-6} DALYs pppy is set by the guidelines as the globally acceptable level of additional disease burden arising from working in wastewater-irrigated fields, or consuming wastewater-irrigated crops. This level of health protection is the same as that used for drinking water in its 4th edition guideline [55]. This also means that the health risk resulting from wastewater exposure in agriculture is the same as those from drinking fully treated drinking water [55, 56].

Box 1.1: The QMRA Approach

Step 1: Hazard Identification

This step describes the acute and chronic or adverse human health effects associated with the hazards or the microbial agent (e.g. *E. coli*, norovirus, rotavirus, *cryptosporidium, Campylobacter*).

Step 2: Exposure Assessment

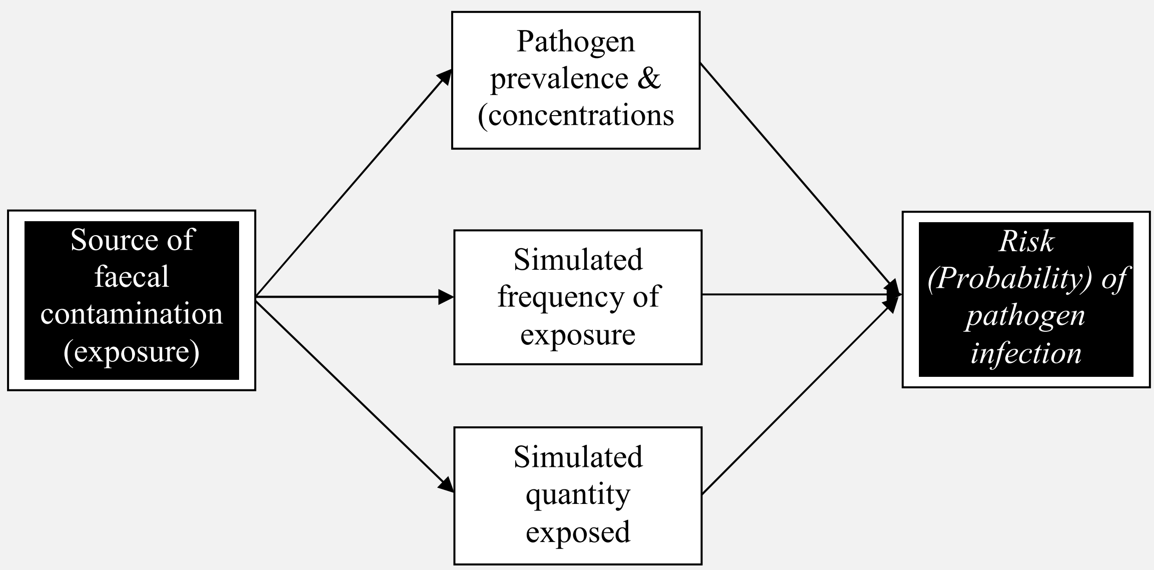
This stage determines the size and nature of the population exposed and the route, amount or concentrations, distribution of microorganisms and duration of the exposure.

Step 3: Hazard Characterization (including Dose-Response Assessment)

The dose response stage of the QMRA provides a quantitative estimate of the risk of response (infection, illness or death) with respect to a known dose of a pathogen. It also translates the estimated dose received by an individual in some 'consuming event' into a probability that the person will make a transition between two health states.

Step 4: Risk Characterization

This last stage of the QMRA process integrates the information from the first three steps to estimate the magnitude of the public health problem or to estimate the incidence in the affected population and to evaluate noteworthy conclusions, variability and uncertainty. It addresses both the qualitative and quantitative features of the assessment as well as the identification of strengths and uncertainties in the assessment. Overall, the estimated risk is based on the pathogen concentrations and the exposure levels as depicted in the diagram below:



This guideline equates to a tolerable disease risk of 10^{-4} pppy, and infection risk of 10^{-3} pppy of rotavirus, the index organism for the viral group. Rotavirus is considered to pose the highest risk and therefore this reduction should provide sufficient protection for bacterial and protozoan infections [21]. Since the guidelines were developed, a norovirus dose response model has also been developed, and there have been suggestions for norovirus to be used instead of rotavirus for health assessment [57]. This, because norovirus is a major cause of viral gastroenteritis in both children and adults worldwide, unlike rotavirus which causes diarrhoea related diseases predominantly in children [22].

Although useful, QMRA depends on the quality of its basic input data that describe the occurrence, persistence and human dose-response to pathogens in the environment [54]. These data are often unavailable or incomplete. Currently, the WHO 'wastewater' QMRA relies on many assumptions from different datasets to support their model as a result of limited primary data on human exposure to faecal pathogens [58, 59]. In most QMRA estimations, simple correlations (mostly linear relationships) of indicator organisms (especially *E. coli*) are used to derive approximate concentrations of actual pathogens. These correlations could, however, lead to overestimation or underestimation of the actual risk and subsequently the required pathogen reductions since actual relationships between indicator organisms and pathogen concentrations are complex (depends on factors such as transport and fate of organisms, regrowth potential, and susceptibility to treatment processes); also these correlations are only based on few studies and hence no strong evidence of the assumed fixed relationships [22, 60]. For example, many pathogens are more resistant to die-off in the source environment than indicators, or have greater resistance to removal by treatment processes, and hence the absence of indictors may not necessarily mean absence of pathogens [61, 62]. For example, *E. coli* has been found to be a bad indicator for Salmonella, and even worse for viruses, protozoa, or helminths.

QMRA studies estimating the risk of consuming wastewater irrigated produce have often made extrapolations based on initial contamination at the farm level without considering market, food vendor or household practices [58, 59, 63]. This approach could be misleading since post-harvest activities could either promote or prevent cross contamination as well as influence microbial multiplication [64]. For example, a study in Pakistan showed that poor hygiene, and environmental conditions were greater risk factors for faecal contamination of produce, than the use of untreated wastewater for irrigation [65]. Again, most studies investigating farmers' occupational risk have often limited the source of contamination to irrigation water [59] despite the high levels of faecal coliforms and helminths eggs in irrigated soil [49, 66, 67]. Moreover, the use of chicken, and other animal manure have been found to be another significant risk factor for soil contamination at wastewater irrigated fields. Wastewater farmers' exposure to faecal matter has also been based on reported days of working in the field, and not their direct contact to wastewater, or contaminated soil; nor are farmer's actual hand-to-mouth contacts included in the model. The above limitations means that the WHO QMRA model for wastewater use in agriculture primarily estimate potential risk, and not necessarily an actual risk. The many assumptions and complexities of the models may also make QMRA models more difficult to evaluate in terms of plausibility and validation [22].

1.5.2 The Multiple-barrier approach

The multiple-barrier approach prescribes good practices, or health protective measures needed to achieve specific health-based targets set out by the WHO guidelines [3]. The approach recognizes health interventions at three levels or disease transmission domains: farm (produce cultivation), market (sales of raw produce) and consumer level (sales and consumption of prepared salad) (Figure 1.2).

Risk management approaches recommended at the farm level include wastewater treatment, controlled wastewater application that limits contact with wastewater (e.g. drip irrigation), crop restriction (e.g. only irrigation of crops consumed cooked), and human exposure control (e.g. wearing protective clothing). At markets and food kitchens, measures include: hygienic practices during sales and food preparation like: produce washing, disinfection and cooking, while improving access to safe drinking water and sanitation especially on farms are also recommended. A combination of these protective measures should ensure that health targets can be achieved in the short, medium, and long term depending on a country's technological, institutional or financial conditions [68].

While these are valuable measures for risk reduction, there is still inadequate field-based evidence on the effectiveness, and uptake of these risk reduction measures especially regarding hygienic food marketing and food preparation at markets, homes and kitchens in low and middle-income countries [10]. For interventions in kitchens, there is a debate on the type, and effectiveness of disinfectants used on salad crops [69]. The debate often dwells on the method of application including the doses, washing times, duration and sequence of application. At farm level, there is limited evidence on the practical application, and uptake of recommended risk reduction measures. For example, in Kenya, a survey showed that less than 15% of farmers actually practiced any of the WHO recommended measures, and only a small proportion (21%) of farmers practiced crop restriction [70]. Similar findings were observed in Ghana, where farmers felt uncomfortable with the use of boots, were unprepared to practice crop restriction for loss of economic returns, and were unwilling to invest in any form of on-farm treatment methods due to cost and land insecurity [49, 71, 72].

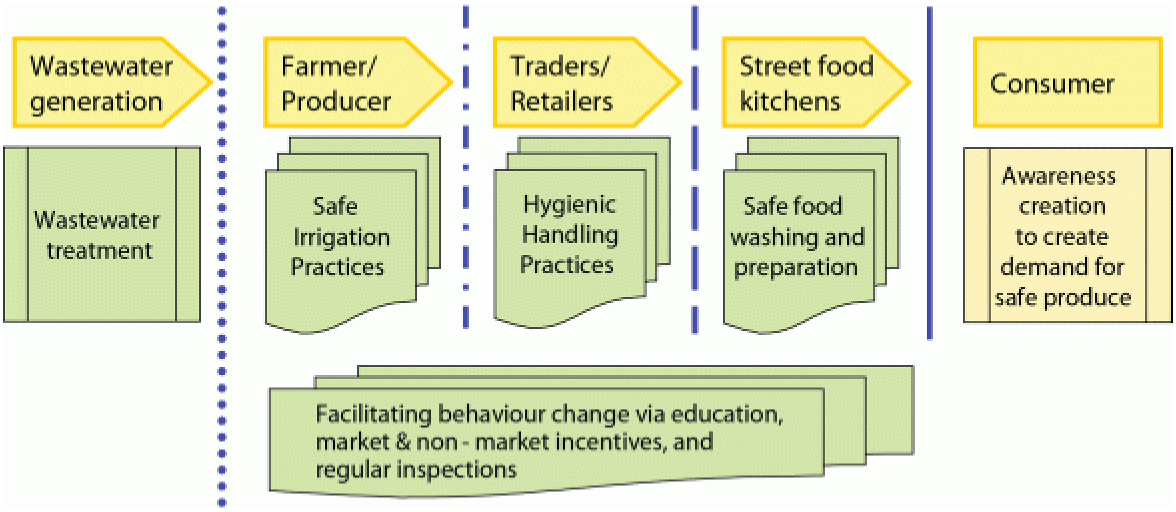


Figure 1.2: WHO Multi-barrier approach for wastewater irrigation (Source: [4])

Besides the low uptake of the non-wastewater protective measures, wastewater has been argued to be more effective in reducing risks at the farm level but not necessarily at the consumer level [73]. It is also seen to address environmental concerns as most wastewater treatment plants are designed based on the need to reduce organic and suspended solids loads to limit pollution of the environment, and rarely on the primary need to remove pathogenic organisms to protect human health [4, 6]. The multi-barrier approach which is based on the Hazard Analysis and Critical Control Points (HACCP) is also perceived to be narrower focused since much attention is on food safety but ignores the broader environmental protection, and other benefits of wastewater treatment [74]. It also requires behaviour change, which can be complicated, and at the same time very limited research has been conducted in relation to wastewater irrigation [72, 73]. Again, the multi-barrier approach will only be adopted on a large scale if at-risk groups prioritise health risk, or become aware of the actual sources of health risks or hazards. Despite the shortfalls of wastewater treatment, and the multi-barrier approach, the use of the two approaches complement each other as in most cases adopting only one approach does not necessarily achieve the health based targets, or treatment objectives [21, 71].

1.6 Microbial hazards considered for this study

Escherichia coli (*E. coli*)

Escherichia coli is a normal inhabitant of the gastrointestinal tract of humans and other warm-blooded animals and is often considered a harmless organism. It is a member of the total coliform group but is considered as the most specific indicator of faecal pollution and is therefore used as a standard to indicate the presence of faecal contamination of environmental and food samples [22, 75]. The presence of *E. coli* is also likely to indicate the presence of other disease causing organisms such as pathogenic bacteria, viruses and protozoans though it is a poor indicator for viruses and protozoans that can survive much longer than the bacteria indicator [75]. However, several strains are pathogenic and can cause gastroenteritis; among these are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) [22]. These strains are grouped by their mechanisms of pathogenesis and are spread by the faecal-oral route of transmission. Pathogenic *E. coli* are known to cause several diarrhoea related diseases including traveller's diarrhoea especially in persons from industrialised countries, chronic childhood diarrhoea and infant diarrhoea. While some of these diarrhoea related diseases can be mild and last up to a period of 5 days, others can also be very severe and prolonged or persistent lasting more than 14 days and with a case fatality rate as high as 50% [22]. EAEC in particular is associated with persistent diarrhoea and is a major cause of illness and death in children. Some victims can also develop haemolytic-uremic syndrome (HUS), which can result in renal failure and haemolytic anaemia or can result in permanent loss of kidney. Most outbreaks of pathogenic *E. coli* have been associated with food such as raw beef and chicken, undercooked or raw hamburger, unpasteurized milk and fruit juices, and vegetables contaminated with cow dung. Waterborne outbreaks occur through nondisinfected groundwater and recreational waters. For example studies have shown that *E. coli* O157:H7 is commonly found in domestic sewage at levels from 10 to 100 CFU/100 ml and in wastewater from slaughter-houses from 100 to 1000 CFU/100 ml [22].

Norovirus

Noroviruses belong to the family of Calicividae and is one of two genera (the other being *Sapoviruses*) that is known to infect humans [22]. They are also nonenveloped viruses with a diameter of approximately 26 to 35 nm and a positive-sense ssRNA genome [76]. Although there are several genotypes of noroviruses, the genotypes GI (NV-GI) and GII (NV-GII) are the most common types identified to cause illness [22]. Noroviruses are major causes of both food and water borne disease and can infect both children and adults. NoVs are the leading cause of food-borne outbreaks of acute gastroenteritis and the most common cause of sporadic infectious gastroenteritis affecting people of all age group [77, 78]. NoV food-borne outbreaks often result from the consumption of shellfish, fresh produce and ready to-eat food contaminated by infected, but possibly asymptomatic, food handlers [79, 80]. Age is a significant factor associated with norovirus infections and related deaths. For example, a review of norovirus infections resulting in 158 associated deaths in 12 countries showed that for age related data, 61% of the deaths were found in those above 65 years of age with 22% and 17% occurring in age groups less than 2 years of age and between 49 and 65, respectively. Norovirus usually produce a mild and brief illness, lasting between one and two days. The disease is characterized by nausea and abdominal cramps, followed commonly by vomiting in children and diarrhoea in adults [22]. Mortality does occur but usually only in immunocompromised individuals or the elderly [81]. The virus is often transmitted by ingestion of food or water contaminated with faecal matter, or by direct or indirect contact and also via aerosol [22]. Norovirus can also be spread by contaminated surfaces or formites (inanimate objects) such as toilet flush handles and doorknobs and are especially common in environments such as schools, hotels, summer camps and hospital emergency rooms [22, 82]). Through epidemiological studies, public toilets have also been shown to be responsible for outbreaks of pathogens including norovirus [22]. The transmission of noroviruses is enhanced by its low infectious dose – fewer than 10 particles could cause infection [57], high resistance to disinfection [83] and long term stability and survival in the environment [82, 84]. NoV is also resistant to many industrial food preservation methods and can survive chilling, freezing, acidification, reduced water activity and modified atmosphere packaging [85]. Additionally, there are reported outbreaks of NoVs in sewage polluted drinking water due to NoVs high resistance to wastewater treatment and high persistence in aquatic environment [86, 87].

Adenovirus

Adenoviruses are double-stranded DNA (dsDNA) icosahedral viruses approximately 70 nm in diameter and belong to the Adenoviridae family of viruses [76]. Adenoviruses cause a wide variety of illnesses in humans from eye infections to diarrhoea. There are about 57 known types of adenoviruses that infect man, with most of these human illnesses associated only with one-third of adenovirus types [22]. Adenovirus types 31, 40, 41, and 52 are known to cause gastroenteritis. The enteric adenoviruses 40 and 41 have also been recognized as the second most important etiological agents of viral gastroenteritis in children [88, 89]. These viruses are also transmitted through the oral-faecal route. Adenoviruses have also been found to have enhanced survival in water, large concentrations in untreated sewage or sewage polluted waters and high resistance to UV light disinfection [22, 90, 91]. Although norovirus was the key virus considered in the health risk assessment for this PhD research, some information on the presence and concentrations of adenovirus in various environmental and food samples (e.g. irrigation water, soil and raw lettuce) that were collected in this study have been included at appropriate sections in this thesis. The objective was to provide researchers and other stakeholders with this information especially for those who are interested and likely to do further research on adenovirus in this research area.

1.7 Gaps in knowledge

Currently it is unknown the number of countries that have adopted the new WHO guidelines, though Ghana appears to be the only country in Sub-Saharan Africa to have captured it in its "National irrigation policy, strategies and regulatory measures" document as a policy implementation strategy. The policy states that institutions should: *"support best practices for the safe use of marginal quality water in accordance with WHO Guidelines for the Safe Use of Wastewater, Excreta and Grey-water in agriculture"* [92]. The shift of the WHO guidelines to QMRA and health-based targets demands that a more data-intensive risk assessments, and identification of critical control points (barriers) are conducted. This also requires that key exposure pathways, practices and behaviours that expose farmers and consumers to faecal pathogens are identified. Besides the identification of critical risk practices and pathways, reliable data on farmers and consumers' exposure frequencies to wastewater, and farm soil are needed to help validate, and improve risk estimates from QMRA models. In order for the multi barrier approach to function it is important to understand what drives farmers, market vendors and consumers, and what risk avoidance methods are practiced, and why, or why not.

Further to the above research gaps, there is limited, or only anecdotal evidence on the uptake of the multi-barrier approach especially the non-wastewater treatment protective measures. Large scale adoption of these measures by farmers and vendors are needed at all stages of the food chain in order to meet performance targets, achieve health based targets and also make interventions more cost-effective [93]. The multi-barrier approach is based on messages and therefore likely to be adopted only when at-risk groups know, and correctly understand these messages but more importantly if they know of the real sources of risk or hazards. Hence, the need for more in-depth formative based studies to thoroughly understand target groups awareness and perceptions about wastewater associated health risks, and factors that define market demand for safer crops or enforced regulations and controls.

1.8 Study Aims and Objectives

The research presented in this PhD thesis had as overall aim to test the appropriateness, and to strengthen where possible, the current QMRA and multi-barrier approach advocated by the WHO guidelines for the safe use of wastewater in agriculture. It achieves this by assessing critical behaviours and exposures associated with microbial quality of salad produce, and the transmission of faecal pathogens in wastewater farmers and consumers at farms, markets, street food vending sites, and restaurants and hotels, as depicted by Figure 1.3.

The specific objectives of the study are:

1. To identify and quantify key exposures and behaviours associated with the transmission of faecal pathogens in farmers using wastewater for irrigation.
2. To describe how produce quality changes from farm to fork, and to identify key risks factors and possible control points for faecal contamination of salad crops in urban agriculture in Accra, Ghana.
3. To assess how farmer, crop handler and consumer knowledge and awareness of wastewater irrigated produce related health risks influence their buying, consumption and food hygiene practices.
4. To estimate the norovirus pathogen infection risk among consumers of wastewater irrigated produce by QMRA using field-based data collected from Accra, Ghana.

1.9 Study Area

The use of wastewater for irrigation is common in Ghana as it is estimated that 40,000 hectares of land are irrigated annually with wastewater, while 2,000 farmers, 5,300 street food vendors and over 800,000 consumers in major cities in Ghana benefit from urban (wastewater) agriculture every day [94]. The largest total area irrigated with wastewater is situated in the capital of Ghana, Accra, with a population of over 1.85 million people as of 2010 [95], and is the place where this PhD research was conducted. In Ghana, adequate provision of sanitation remains very low with improved sanitation coverage at 14% [96], while less than 5% of all households are connected to piped sewerage systems that are linked to sewage treatment plants [95]. In the Greater Accra Metropolitan Area (GAMA), 97% of all public-owned and public-managed sewage treatment plants are non-functional [97]. This has resulted in the common practice of disposing faecal waste into the ocean, and other water bodies without treatment [97].

In Accra, an estimated 160 ha of agricultural land are irrigated annually with either untreated, or partially treated wastewater, while an additional 4,600 ha or even more could be irrigated if 10% of the 280 million m^3 of wastewater produced by urban Ghana are used [98, 99]. Farmers in Accra use untreated municipal wastewater sources such as drain water (combined sewers), dug-outs (ponds), and streams that function like a drain to cultivate vegetables, which include: lettuce, spring onion, cabbage and a variety of other local vegetables. The types of wastewater used by farmers for vegetable irrigation comprise of a mixture of wastewater from residential areas (greywater and blackwater), from institutions and offices, from car washing bays or from hospital buildings and apartments (Photo 1.2). Irrigation is commonly done manually using watering cans. Vegetables grown on wastewater are sold at local wholesale, and consumer markets, but are also bought directly by restaurants and street food vendors.

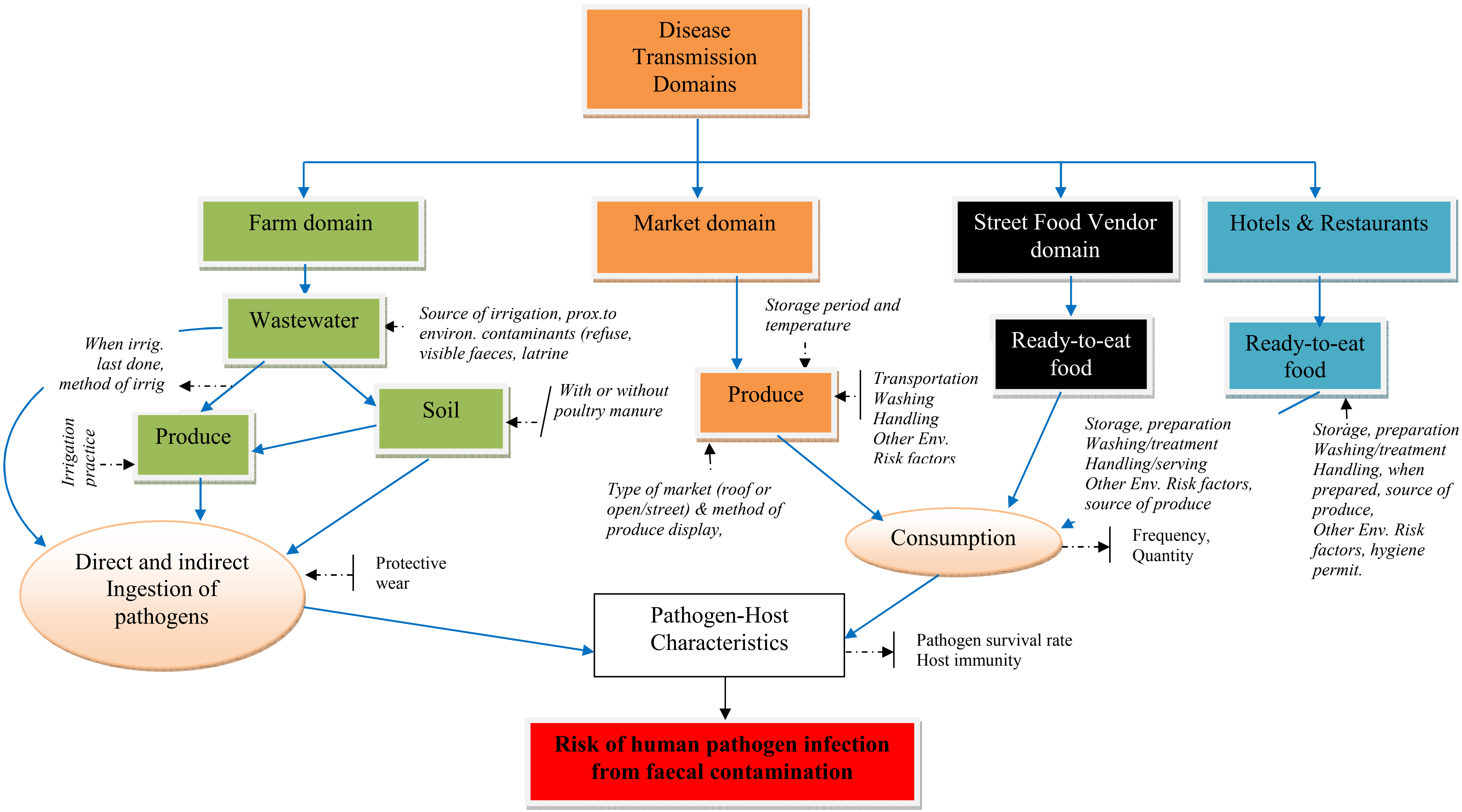


Figure 1.3: Generalised Pathways for assessing risk of human pathogen infection from faecal contamination along the farm-to-fork

Photo 1.2: Irrigation water sources at farm sites in Accra, Ghana

1.10 Thesis outline

Apart from Chapters 1 and 6, each of the Chapters addresses one of the four study objectives. Chapter 2 presents the results of a faecal exposure assessment of farmers irrigating with wastewater. The study aimed to identify critical pathways for faecal disease transmission and quantify farmers' contact time to irrigation water and contaminated soil. The collected data was used in the QMRA model developed for the WHO guidelines on safe use of wastewater in agriculture, to assess if practices met guideline standards.

In Chapter 3, a farm to fork assessment is presented that aimed to identify key risk factors for produce microbial contamination from wastewater irrigated farms, to markets, street vending sites and restaurants. In Chapter 4 a study is presented that assesses, how knowledge, awareness and risk perceptions of wastewater farmers, market salespersons of produce and consumers of salad influenced their actual practices, consumption of salad produce, and their adoption of health protective measures. In the final result Chapter, Chapter 5, estimates of pathogen infection risks from direct and indirect exposure to wastewater and wastewater irrigated products are presented by modelling observed behaviour data and microbial data using a QMRA model approach. The last section of the thesis (Chapter 6) discusses the key findings from the different results Chapters with the aim to highlight the additional evidence needed to strengthen the current QMRA and multi-barrier approach as advocated by the WHO for the safe use of wastewater in agriculture.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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SECTION A – Student Details

Student	Prince Antwi-Agyei
Principal Supervisor	Dr Jeroen Ensink
Thesis Title	Wastewater use in urban agriculture: an exposure and risk assessment in Accra

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	**Water Research**
Please list the paper's authors in the intended authorship order:	Prince Antwi-Agyei, Adam Biran, Anne Peasey, Jane Bruce, and Jeroen Ensink
Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	My supervisor and I designed the study. I developed all data collection tools and collected field data. I also performed all statistical analysis and was the lead author for the manuscript.

Student Signature: ____________________ **Date:** ______________________

Supervisor Signature: __________________ **Date:** ____________________

Chapter 2: A faecal exposure assessment of farm workers in Accra, Ghana

Photo 2.1: A farmer in Accra, Ghana, using watering cans to fetch irrigation water from a dug-out/pond.

Abstract

Wastewater use in urban agriculture is common as a result of rapid urbanisation, and increasing competition for water. In order to minimize the health risks to agricultural workers and consumers of wastewater irrigated produce the World Health Organization (WHO) has developed guidelines for the safe use of wastewater in agriculture. These guidelines are based on a Quantitative Microbial Risk Assessment (QMRA) model, though the reliability of this model has been questioned due to a lack of primary data. This study aimed to assess the ability of the WHO guidelines to protect farmers' health, by identifying and quantifying key exposures associated with the transmission of faecal pathogens in wastewater irrigated agriculture.

Eighty farmers were observed and interviewed during the dry and wet seasons and water and soil samples were analysed for the presence of *E. coli*, human norovirus and adenovirus. The results showed that irrigation water and the use of poultry manure were key risk factors for soil contamination. Although irrigation water was significantly more contaminated than farm soil (5.0 Log *E. coli*/100 ml vs. 2.2 Log *E. coli*/g), exposure to farm soil was found to be the key risk pathway. During the observations 93% of farmers worked barefoot, 86% experienced hand-to-soil contact, while 53% experienced hand-to-mouth soil events, though there was a lack of water to mouth contacts. On average, farmers were found to have 10 hand-to-mouth events per day. From the indicator based QMRA model the estimated norovirus infection risk to farmers was found to be higher than guidelines set by the WHO. The study recommends the incorporation of hand-to-mouth events, the use of actual pathogen concentrations, and the use of direct exposure frequencies in order to improve the reliability of risk estimates from QMRA models.

Keywords:

Wastewater use, faecal exposure assessment, urban agriculture, farmers, Ghana

2.1 Introduction

The use of untreated, or partially treated wastewater in agriculture is common in countries with rapid and uncontrolled urban growth [21]. The exact extent of wastewater use in agriculture is unknown, but estimates range from between 4 to 24 million ha of agricultural land receiving wastewater [8, 100, 101]. The use of wastewater for irrigation has been associated with health risks in farmers, and consumers of wastewater irrigated produce [31]. In order to minimize these health risks, the WHO has developed guidelines for the safe use of wastewater in agriculture. These guidelines have been the focus of discussion for years, and have seen several revisions with the most recent guidelines published in 2006 [21].

The current guidelines are based on a Quantitative Microbial Risk Assessment (QMRA) and use a similar approach to the WHO drinking water guidelines [56]. QMRA is used to estimate disease risks that should not exceed the maximum permissible disease burden of 10^{-6} DALY per person per year (pppy) arising from exposure to wastewater [21]. The risk estimate from QMRA is then used to determine the total pathogen reductions required to achieve the tolerable risk of infection due to a particular pathogen. Although useful, the reliability of estimates from QMRA depends on the quality of the input data describing the occurrence, persistence, human dose-response of pathogens in the environment, and on the time exposed to sources of hazards.

The concern with the wastewater guidelines QMRA is that they are based on many assumptions from different datasets, and as a result lack data on actual concentrations of pathogens in wastewater and contaminated soil [59]. In the WHO QMRA model for assessing wastewater health risk, various correlations or ratios of *E. coli,* or faecal coliforms to pathogens are used instead of actual concentrations of pathogens [102]. In addition most QMRA studies on occupational risk to farmers rely on approximations for the frequency, duration, and type of contact by farmers irrigating with wastewater. In the QMRA model, the number of days a farmer works in the field over a year equates to the number of days they are likely to accidentally ingest contaminated soil, though there is lack of evidence to support this. In addition the QMRA model assumes only a faecal-oral transmission route for pathogens in wastewater contaminated soil, not for exposure to water, nor for direct contact with contaminated soil, even though other modes of transmission are well established [103-105]. The paucity of data for risk assessment therefore calls for more field-based data that can help validate, and improve the accuracy, and reliability of risk estimates from QMRA models. This paper presents the results of an exposure assessment which observed farmers' exposure to wastewater, and wastewater irrigated soil in Accra, Ghana as part of their day-to-day farming activities. The study aimed to determine key exposures associated with the risk of faecal disease transmission in farmers using wastewater for irrigation.

2.2 Methods

In the period from October to December 2012 (dry season), and from June to August 2013 (wet season) farmers irrigating with wastewater in Accra, Ghana were observed and interviewed to identify risk behaviours, and to quantify their contact time to faecal pathogens. In addition water and soil samples were collected and analysed for *E. coli*, human adenovirus and norovirus.

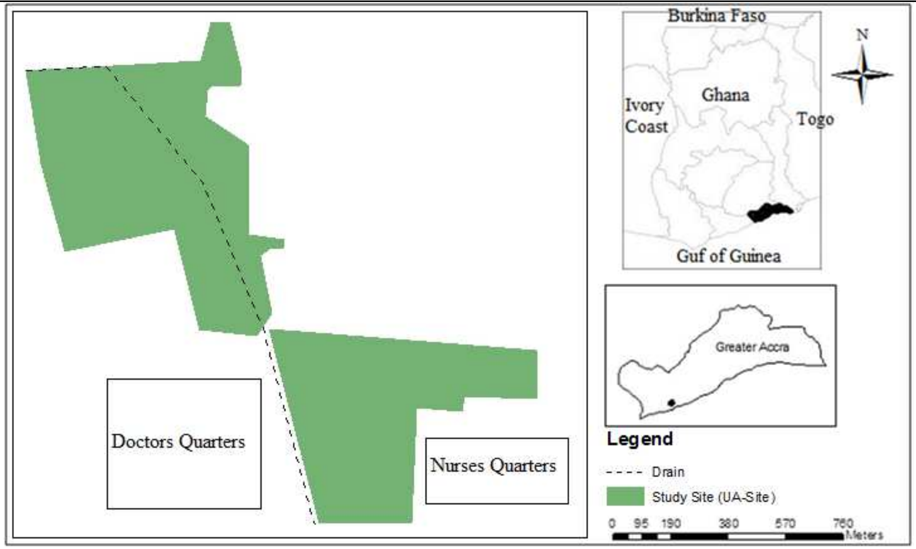
2.2.1 Study Area

Accra is the capital city of Ghana with an estimated population of 1.85 million [95]. It is estimated that less than 6% of Accra is connected to a sewerage system, with the majority of the city reliant on onsite forms of sanitation like: pit latrines and septic tanks [106]. There are over twenty wastewater treatment facilities in Accra, but only seven were reported to be functioning adequately [106]. There are seven major sites where wastewater in agriculture is used, with a total area of 160 ha [99]. Farmers at these sites apply water through watering cans using irrigation water sources that include drain water, channelled rivers and dugouts. The dugouts are man-made ponds used to store the various sources of water used for irrigation. The most commonly cultivated crops are salad vegetables including: lettuce, cabbage, spring onions, and local vegetables. Three of the sites (Korle Bu, Dzorwulu and Marine Drive) were selected for this study because of their large size, the cultivation of salads crops normally consumed uncooked, and the use of wastewater for irrigation.

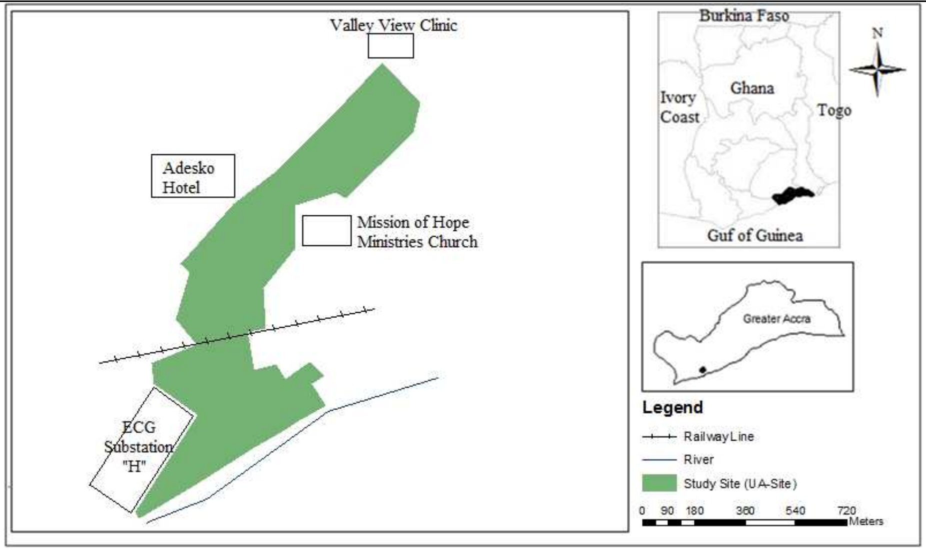
2.2.2 Data Collection

Sample collection and analyses

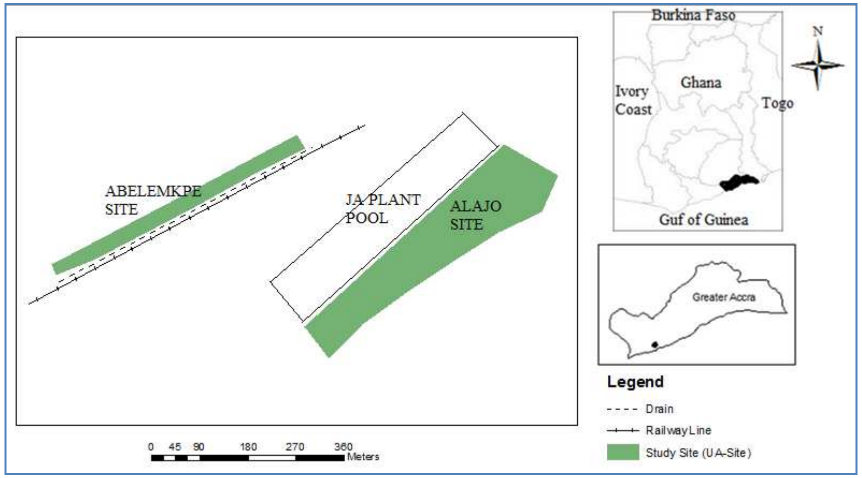
Irrigation water and soil samples were collected between 7 am and 10 am. Irrigation water samples were collected directly from open drains, and dug-outs into sterile 500 ml Whirl-Pak bags using a sterile bailer. Samples were collected from where each of the 80 farmers was observed collecting water for irrigation.



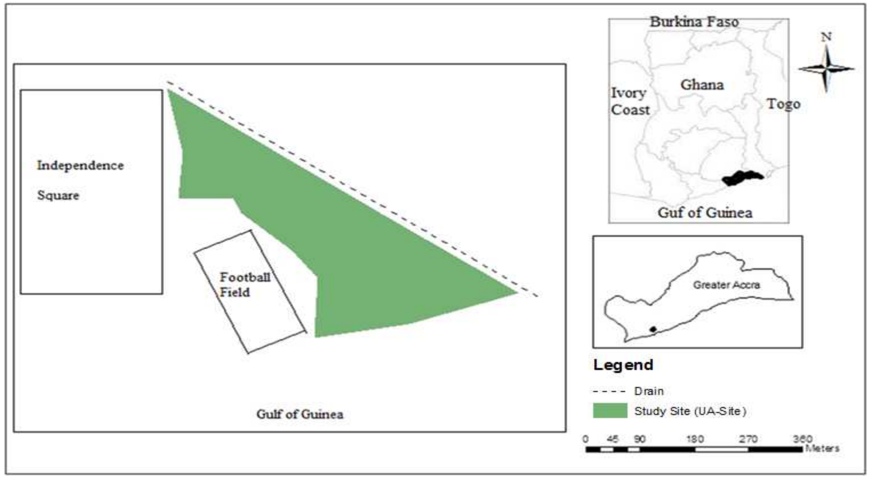
UA-site at Korlebu



UA-site at Dworwulu



UA-site at Plant Pool (For pilot study)



UA-site at Marine Drive

Figure 2.1. Map of Urban Agriculture sites in study communities

For all samples, the site conditions including exposure to visible human faeces, and proximity to refuse and latrines were recorded using a structured observation sheet or sample collection forms (Annexes 1a & 1b). At the laboratory serial dilutions of the raw sample with phosphate buffered saline (PBS) were prepared in sterile micro centrifuge tubes.

Farm soil samples were collected using a sterile metal spatula to a depth of 5 cm into 250 ml Whirl-pak bags until the bag was at least half full. A total of 7 soil samples were collected within an area of 3 m^2, and combined into a single sample to increase sample representativeness [107]. All collected samples (including irrigation water samples) were placed in an ice-box, and transported to the laboratory within 6 hours of collection for processing. Samples were processed immediately, or stored in a 4^oC refrigerator until ready for processing within 24 hours. At the laboratory, soil samples were homogenised, and 10 g of the sample was measured to which 20 ml of sterile PBS (pH of 7.2) was then added. The sample was vortexed for 30 seconds and shaken vigorously on a shaker for 30 minutes at room temperature. The sample was then allowed to settle for 15 minutes, and 10 ml of supernatant transferred into a new sterile 50 ml tube for the *E. coli*, noro and adenovirus assays. RNA was extracted using the Qiagen Viral extraction kit (Qiagen, Venlo, The Netherlands), and DNA using the MPBio FastDNA kit for Soil (MP Biomedicals, Santa Ana, USA). Virus presence/absence and inhibition in water and soil was determined using Quantifast Pathogen IC RT-PCR and PCR kits [108]. Norovirus and adenovirus concentrations were determined using Qiagen OneStep kits [108]. All laboratory staff were blinded on the sources of irrigation water, and farm soil in order to eliminate potential biases during sample analyses. Soil and water samples were analysed using the membrane filtration technique with BBL MI agar (Beckton Dickinson, Sparks, USA) to determine the prevalence and concentrations of *E. coli* [109]. Serial dilution ranges were pre-optimized to ensure that ranges allowed enumeration of roughly 95% of samples, per sample type.

The number of soil and irrigation water samples to be collected was calculated with STATA 12 (StataCorp LP, College Station, USA) and corresponded to the estimated number of farm produce samples to be collected at the farm as part of a farm to fork study (Chapter 3). Sample size for produce was estimated using a hypothetical detectable difference in mean faecal coliform concentration levels of 5% to 10% between produce at farm gates and those at markets based on a related study in Ghana [48]. This resulted in 80 produce samples from 80 different farm plots/beds, and hence 80 each of irrigation water and soil samples were collected in each season. The site conditions including proximity to visible faeces and latrine, and other information were also recorded for each farm sample collected (Annex 1c).

Observations

At the farm sites only farmers who had at least one bed of ready-to-harvest lettuce were included in the study. Eighty farmers were randomly selected using their farm plots/beds as identification. This study used a direct observation approach where researchers observe and record the behaviours as, and when, the behaviours occurred [110]. In as much as possible, observers tried to be unobtrusive during the observation to minimise the effect of their presence on participant's behaviours. Farmers were told that the observations were designed to learn more about their farming activities including irrigation, manure application and "forking" (the use of hand-held knife/fork to turn over the soil to allow air flow, Table 2.1). Farmers were not told specifically that it was meant to document risk behaviours associated with faecal exposure.

Table 2.1. Operational definition of farming activities

Farm activity	**Operational definition**
Bed preparation	The use of hoe, rake and other farm implements to prepare a plot of ground or the soil (farm bed) for planting seedlings of salad crops.
Transplanting	The removal of seedlings from the nursery to be planted on the newly prepared beds
Weeding	The use of hands to remove small weeds that have mixed with the salad crops
"Forking" (soil tilling)	The use of hand-held knife/fork to turn over the soil to allow air flow. This activity is also often done alongside weeding.
Irrigation	The use of watering cans (in some cases water hoses) to irrigate salad crops. Irrigation water sources include drains, dugouts and channelled rivers/streams
Manure application	Application of chicken manure with or without the use of protecting clothing such as hand gloves

A structured observational guide (Annex 4a) was used to record behaviours, while tally sheets were used to capture hand-to-mouth/face contact events. In this study, exposure to faecal contamination was defined as coming into direct contact with either contaminated soil, or irrigation water, or both (by hands, feet or mouth/face) without any protective clothing. Exposure to wastewater was defined as having direct contact (getting wet) with irrigation water. Similarly, touching of the mouth/face during work in the field was defined as accidental contact. Each farmer was observed continuously for three hours (7.00 am – 10.00

am) from October to December 2012. The total time farmers spent on each farm activity was recorded by indicating the start and end times. Similarly, the total time farmers worked unprotected, and came into contact with either soil or irrigation water was recorded. Farm workers access to safe drinking water and sanitation facilities were also observed as well as their food hygiene practices including hand washing.

Questionnaire

Following the field observation, a questionnaire (Annex 2a) was administered to each farmer to gather background information (including personal characteristics), the time and days spent undertaking different farm activities during the rest of year, and the use and application of other forms of fertilizer. Information was also collected on access to water supply, sanitation and hygiene at the farm.

Quantitative Microbial Risk Assessment (QMRA)

The Microsoft Excel QMRA model developed by Mara and Sleigh [102] for the WHO guidelines was used to estimate the pathogen infection risk using the estimated observed and reported exposure frequencies of farmers in this study. In the WHO model, an exposure of 300 days per year is used (although flexible) for labour intensive agriculture; representing farming practices in low and middle-income countries. The model further assumes that between 10 to 100 mg of soil is accidentally ingested by farmers per day during their fieldwork [3, 102]. The model uses the Karavarsamis-Hamilton method [111], together with the norovirus dose-response model by Teunis *et al*. [57] and 10,000 Monte Carlo simulations to estimate infection risk among wastewater farmers for restricted irrigation. A maximum tolerable additional disease burden of 10^{-6} disability-adjusted life year (DALY) loss per person per year (pppy) used in the WHO guidelines was adopted in this study, which equates to a maximum permissible infection risk of 1.4×10^{-3} pppy for norovirus which is considered to pose the highest risk compared to bacteria and protozoans. The estimated risk in this study was compared to a more relaxed DALY loss of 10^{-4} pppy as recommended by Mara *et al*., [112], which also equates to a tolerable norovirus infection risks of 0.14 pppy. These tolerable risks were calculated based on a DALY loss per case of 9×10^{-4} per case of Norovirus (NV) disease [113] and an NV disease/infection ratio of 0.8 [114].

2.2.3 Data Analysis

Data analysis was done using STATA 12, the Microsoft Excel QMRA model developed by Mara and Sleigh [102] and @Risk 6 (Palisade Corporation, NY-USA). All *E. coli* concentrations were Log_{10} transformed for calculations of medians, and inter quartile range (IQR) for irrigation water samples and means, standard deviations and 95% confidence intervals (CI) for soil samples. Unlike irrigation water, *E. coli* concentrations in soil were normally distributed after log transformation. The Mann-Whitney and Kruskal-Wallis tests were used to test the association of different risk factors with irrigation water quality. One-way anova and two sample t-test were used to assess the effect of risk factors on farm soil contamination. Only factors that were significant at 10% in the univariable analysis were included in a multiple regression model that was used to identify risk factors for soil contamination. Statistically significant differences between exposures and outcomes in the multiple regression model were measured at 5% significance level using likelihood ratio test. Irrigation water was also reclassified as a binary variable (≤ 3 Log *E. coli*/100ml and > 3 Log *E. coli*/100ml) representing the old water quality guidelines set by the WHO [27]. The proportion of time farmers worked unprotected for each farm activity was determined as a proportion of the time they work unprotected over the total time used to undertake the activity. The observed annual contact time for each type of contact (e.g. feet-to-soil), was calculated as the product of the total daily contact time for all observed farm activities, the number of days farmers work within a month and the months they work in a year. The expected dose of *E. coli* likely to be ingested due to hand-to-mouth events was estimated from a Poisson distribution [22] using the soil quality from this study and the amount of soil (10 - 100 mg/d) accidentally ingested by farmers. The total observed time farmers were in direct contact in the fields were estimated by assuming 100% feet-contact with soil.

2.2.4 Ethical

Ethical approvals were received from the Ethical Review committees of the London School of Hygiene and Tropical Medicine (LSHTM, reference – 6236), and the Noguchi Memorial Institute of Medical Research in Ghana (Reference – DF22). The study was explained to, and agreed by local leaders and written informed consent was obtained from each individual who participated in the study.

2.3 Results

2.3.1 Irrigation water and farm soil quality and risk factors

During the survey, only 7% of the 160 irrigation water samples, and 9% of the 163 soil samples were found to be free from *E. coli*. Overall, the median concentration of irrigation water was 5.6 Log *E. coli*/100 ml, while the mean concentration of soil was 2.3 Log *E. coli*/g. Drain water was the most contaminated with median concentration of 6.6 Log *E. coli*/100 ml (Table 2.1).

There were 9% and 15% of irrigation water samples that tested positive for norovirus GI and GII respectively, while 47% of the samples were positive for adenovirus. Among the viruses, norovirus GI had the highest concentrations (6.67 ± 0.53 Log gene copies/100 ml) in irrigation water samples, followed by NV-GII, (6.49 ± 0.77 Log gene copies/100 ml), and adenovirus, (5.26 ± 0.54 Log gene copies/100 ml). No soil sample was found to be positive for norovirus genome I (NV-GI) while only 2.5% (N = 79) and 2.7% (N = 75) of the samples were positive for NV-GII and adenovirus, respectively. Mean concentration of adenovirus in positive soil samples was 3.76 ± 0.37 Log gene copies/g.

The use of poultry manure, the type and quality of irrigation water, and the last time soil was irrigated were all associated with increased levels of soil contamination in the univariable analysis (Table 2.1). In the multivariable analysis, the effect of irrigation water and seasonality remained strongly associated with the levels of soil contamination after controlling for the use of chicken manure. There was interaction between seasonality and irrigation water quality which was significant ($p = 0.02$, 95% CI = -0.36, -0.04) in the multivariable analysis and resulted in higher levels of soil contamination in the dry season than in the rainy season (Table 2.1). For irrigation water, univariable analysis showed significant differences in the *E. coli* quality among the different types of irrigation water namely drain water, dug-outs and piped water ($p < 0.001$, Table 2.1). Similarly, irrigation water sources within 3 m radius of refuse was significantly more contaminated than those further than 3 m ($p = 0.02$).

Table 2.2:*E. coli* contamination of irrigation water and farm soil

Water quality	N	Median	IQR***	P_1
E. coli (Log10/100ml)				
Dry season	80	5.37	3.61, 6.27	0.35
Rainy season	80	5.73	3.48, 6.61	
Water sources				
Drain water	36	6.61	5.93, 6.81	<0.001
Dug-out	41	3.78	3.00, 5.69	
Piped water	3	2.65	2.65, 3.30	
Proximity to refuse				
≤ 3m	59	5.90	3.70, 6.72	0.02
> 3m	21	4.57	3.00, 5.79	
Farm soil parameter	**N**	**Mean (SD*)**	**95% CI****	**P_2**
E. coli(Log10/g)				
Dry season	83	2.25 (0.93)	2.05, 2.46	0.93
Rainy season	80	2.24 (0.92)	2.04, 2.45	
With manure (both seasons)				
Yes	128	2.34 (0.89)	2.19, 2.50	0.01
No	33	1.90 (0.94)	1.57, 2.23	
Irrigated with:				
Drain water	36	2.84 (0.61)	2.63, 3.04	<0.001
Dug-out	41	1.79 (0.86)	1.52, 2.06	
Piped water	3	1.27 (0.53)	-0.06, 2.59	
When irrigated (n=80)				
≤ 1 day	32	2.58 (0.90)	2.26, 2.91	0.01
b/n 1 day – 2 days	13	2.11 (0.83)	1.61, 2.61	
> 2 days	35	1.98 (0.90)	1.67, 2.29	
Multivariable analysis				
Exposure	**N**	**Change in mean**	**95% CI****	**P_3**
Irrigation water	160	0.41	0.30, 0.52	< 0.001
Manure	163	0.23	-0.11, 0.57	0.03
Seasonality	160	0.97	0.11, 1.85	0.05
Season #irrigation water	160	-0.20	-0.36, -0.04	< 0.001

SD standard deviation*
*95% CI** 95% confidence interval*
*IQR*** = Interquartile range*
P_1, *p-value calculated using Mann-Whitney test or Kruskal-Wallis test for irrigation water quality.* P_2, *p-value calculated using t-test and Anova for farm soil quality.*
P_3, *p-value calculated using likelihood ratio test.*

2.3.2 Farmer observations

Of the 80 farmers 95% were male, with an average age of 40 years (range 22 – 72). Agriculture formed the main source of income for the large majority of farmers (80%), while over 70% of farmers were literate. There were no toilet facilities found on the sites, and 73%

of farmers reported that they practiced open defecation when working at their fields (Table 2.2). The majority (77%) of farmers ate their food in their fields and mostly consumed it cold. A total of 11 farming activities were observed during the 3 hours, though the majority (79%) of time was spent on 5 key activities, with irrigating (33% of total time) the most common (Figure 2.1). During the observation period, almost all (97%) farmers were observed to have hand-to-soil contact, and 89% of farmers were found to work bare-foot in their field for any of the activities (Table 2.3). In addition, over 90% of farmers involved in irrigation had their hands and feet exposed to irrigation water. The number of hand-to-mouth contacts per farmer was highest during "forking" with an average of 4 events, but ranged from 1 to 12 events (Figure 2.2). For all activities observed within the 3 hours, 86% of farmers experienced hand-to-soil contact for an average time of 100 minutes (55% of total time) while 93% of farmers worked bare-foot for 145 minutes (81% of total time). In addition, 63% of farmers had both feet and hands exposed to irrigation water for at least 88 minutes (49% of total time).

2.3.3 Reported exposure frequencies and risk practices

Farmers reported to work on average of 7.1 hours per day, 28.2 days per month and 11.8 months per year on their farm (Table 2.2). These then translate to an average annual time spent working in the field of 337 days, or 2,393 hours. In terms of farming activities, farmers spend a median time of 720 hours per year irrigating, and a maximum of 2,880 hours per year (120 days), though irrigation was done on average 324 days (27 days per month) per year (Table 2.4). In addition, farmers spend a maximum of 135 hours per year (6 days/year) applying chicken manure (Table 2.4). Of the five major farm activities, irrigation recorded the highest feet-to-soil contact of 88 min/3h (Figure 2.3) which translated into annual median contact of 1,278 h/y (Table 2.3). The observed median feet-to-soil contact for farm activities was 2,002 h/y (maximum of 2,556 h/y, or 107 d/y). "Forking" had the highest hand-to-soil contact time (53%, 152 h/y) while no hand-to-soil contact was observed during irrigation. Every day, farmers had a median of 10 hand-to-mouth events (3,181/y), with the greatest number of events occurring during "forking" (Table 2.3).

Although all farmers reported to wash their hands before eating, only 81% of farmers were observed washing their hands before eating, and only one (6%) was found to use soap (Table 2.2). The use of poultry manure was common, though significantly higher among farmers in the dry season than in the rainy season (Table 2.2). Only a few farmers (6%) were seen using gloves, or masks (1%), though a slightly higher proportion of farmers reported that they used gloves and masks while preparing chemicals for spraying crops (9% vs. 6%). Irrigation cessation was not reported to be practiced, though the average irrigation frequency went down to every other day (56 h) during the rainy season (Table 2.2). Apart from farmers who worked on the farm, other people, especially school children, were observed to use the farm sites as footpaths.

Farmer collecting irrigation water with watering can with hands exposed to water

Farmer planting spring onion with both hands and feet exposed to contaminated soil

A farmer applying poultry manure without protecting clothing (e.g. hand gloves)

"A farmer undertaking forking (turning over of soil) while eating corn (hand-to-mouth contact)

Photo 2.2: Risky farming practices and behaviours

Table 2.3: Characteristics at farm sites

Exposure	N (%)
Poultry manure use*	
Dry season (n = 80)	48 (60)
Rainy season (n = 83)	82 (99)
Last irrigated (N = 80)	
≤ 1 day	32 (40)
1 to 2 days	13 (16)
2 to 3 days	21 (26)
> 3 days	14 (18)
Defecation practices of farmers	
Public toilet	20 (25)
Neighbour's toilet	2 (2.4)
Open Defecation	58 (73)
Source of farmers drinking water	
Sachet water	58 (73)
Piped water	22 (27)
What used to wash hands before eating (N = 76)	
Irrigation water only	2 (2.6)
Piped water only	22 (29)
Sachet water only	8 (11)
Water and soap	44 (58)
Observed hand washing practices before eating (N = 21)	
Washed hands before eating	17 (81)
Washed hands with water and soap	1 (5.9)
Washed hands with only water	16 (94)
Whether drain water increases farmers income compared to piped water	
Yes	48 (60)
No	3 (4)
Cannot tell	29 (36)
Farming as main source of income	
Yes	63 (79)
No	17 (21)
Where farmers eat often when at work (N = 77)	
On the farm	59 (77)
At vending sites	8 (10)
At home	10 (13)
Reported working times in the field	
Average daily working hours (min – max)	7.1 (4, 13)
Average days worked per week (min – max)	6.7 (5, 7)
Average days worked per month (min – max)	28.2 (20, 30)
Average months worked per year (min – max)	11.8 (9, 12)
Average days worked per year (min – max)	336.7 (240, 360)
Average time (hrs/d) spent outside home between 6am and 7pm	11

** p-value, < 0.001*

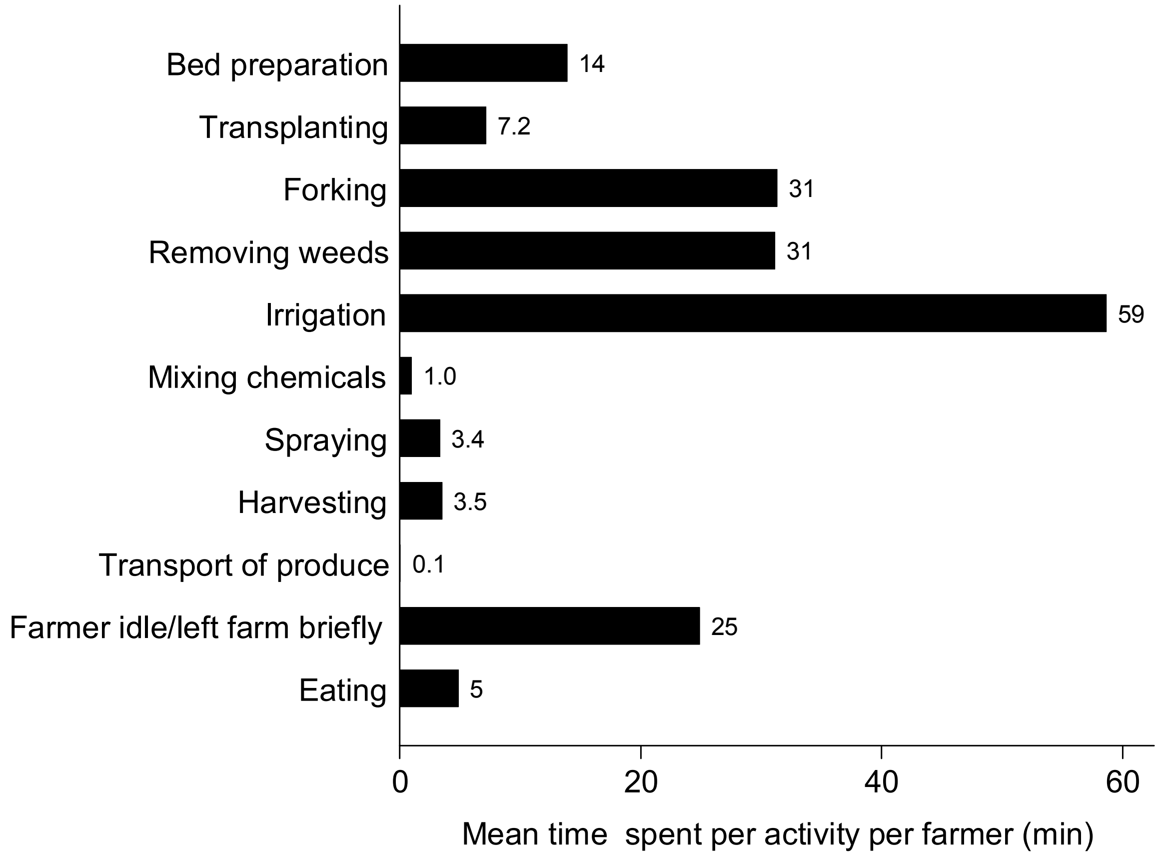


Figure 2.2: Observed time (3 hours) for undertaking farm activities

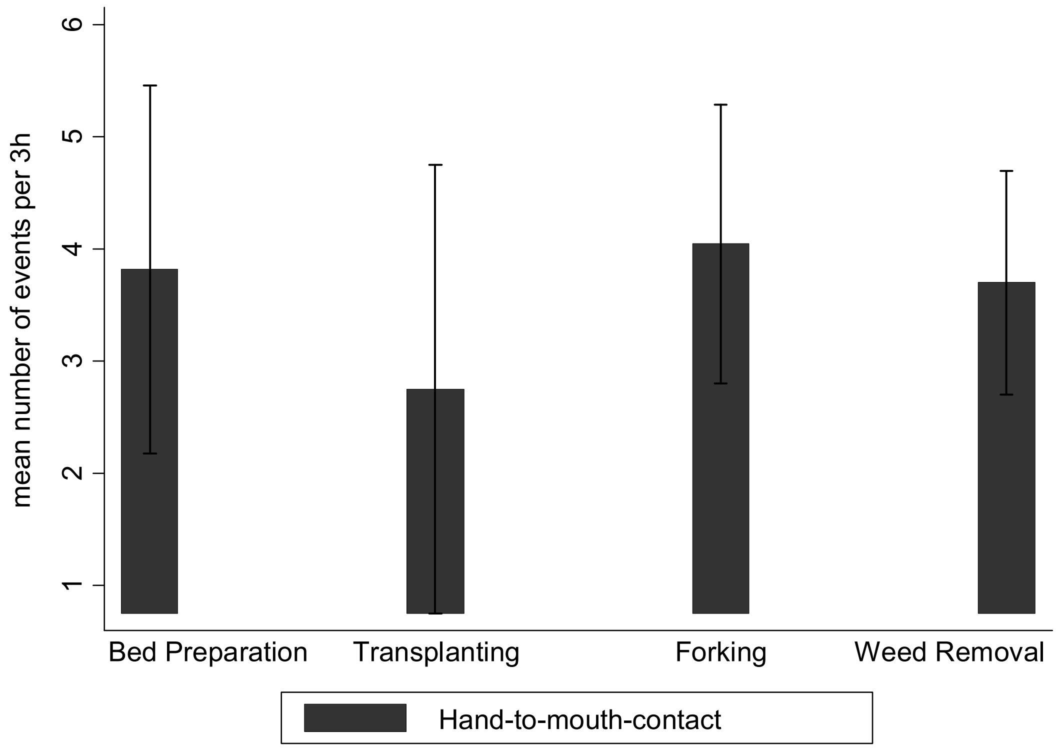


Figure 2.3: Observed farmers' hand-to-mouth contact events per 3 hour observation period, by farm activity.

* *Error bars represent 95% CI of the mean.*

Table 2.4: Farmers' annual contact time to irrigation water and farm soil per contact type and farm activity

Variable/Parameter	% of farmers involved in activity at peak period % (N = 80)	Percentage of farmers with contact to faecal contamination, % (n)	Median (IQR) contact time, h/y
Bed Preparation (n = 19)	24 (19)		25 (16, 39)
Hand-to-soil		100 (19)	24 (14, 39)
Feet-to-soil		90 (17)	24 (16, 33)
Hand-to-mouth/face, nr/y*		58 (11)	85 (45, 227)
Transplanting (n = 11)	14 (11)		18 (13, 45)
Hand-to-soil		100 (11)	18 (13, 45)
Feet-to-soil		100.(11)	18 (13, 45)
Hand-to-mouth/face, nr/y		36 (4)	85 (57, 270)
Soil tilling (Forking, n = 36)	45 (36)		150 (83, 290)
Hand-to-soil		97 (35)	152 (84, 308)
Feet-to-soil		92 (33)	144 (81, 273)
Hand-to-mouth/face, nr/y		61 (22)	454 (227, 852)
Weed Removal (n = 42)	53(42)		99 (47, 189)
Hand-to-soil		100 (42)	99 (47, 189)
Feet-to-soil		98 (41)	95 (47, 189)
Hand-to-mouth/face, nr/y		48 (20)	256 (128, 852)
Irrigation (n = 55)	69 (55)		1113 (426, 1617)
Hand-to-irrigation water		93 (51)	1278 (451, 1633)
Feet-to-soil		89 (49)	1278 (450, 1633)
Feet-to-irrigation water		91 (50)	1295 (451, 1633)
Total hand-to-soil contact**	100 (80)	86 (69)	1339 (909, 1732)
Total feet-to-soil contact†	100 (80)	93 (74)	2002 (1625, 2300)
Total hand-to-mouth contact events	100 (80)	53 (42)	3181 (1704, 5964)

nr/y = number of events per year*
*** Total hand-to-soil contact for 5 farm activities – bed preparation, transplanting, soil tilling, weed removal and harvesting*
† Total feet-to-soil contact for 8 farm activities - bed preparation, transplanting, soil tilling, weed removal, irrigation, spraying, harvesting and transport of produce to roadside.

Table 2.5: Farmers' reported annual working time per farm activity

Farm Activity	Farmers	Average frequency of activity, d/m	Median (IQR) (h/y)	Min – Max (h/y)
Bed preparation	79	1.1	24 (12, 36)	2.25, 192
Transplanting	79	1.1	27 (18, 48)	3, 240
Soil tilling ("Forking")	80	6.9	180 (96, 219)	24, 528
Removing weeds	80	5.6	96 (48, 174)	12, 720
Irrigation	80	27.0	720 (360, 1080)	72, 2880
Poultry manuring	79	1.3	12 (9, 24)	3, 135
Total time for 6 activities	79	NA	1062.1 (771, 1634.4)	282, 3396

NA = not applicable

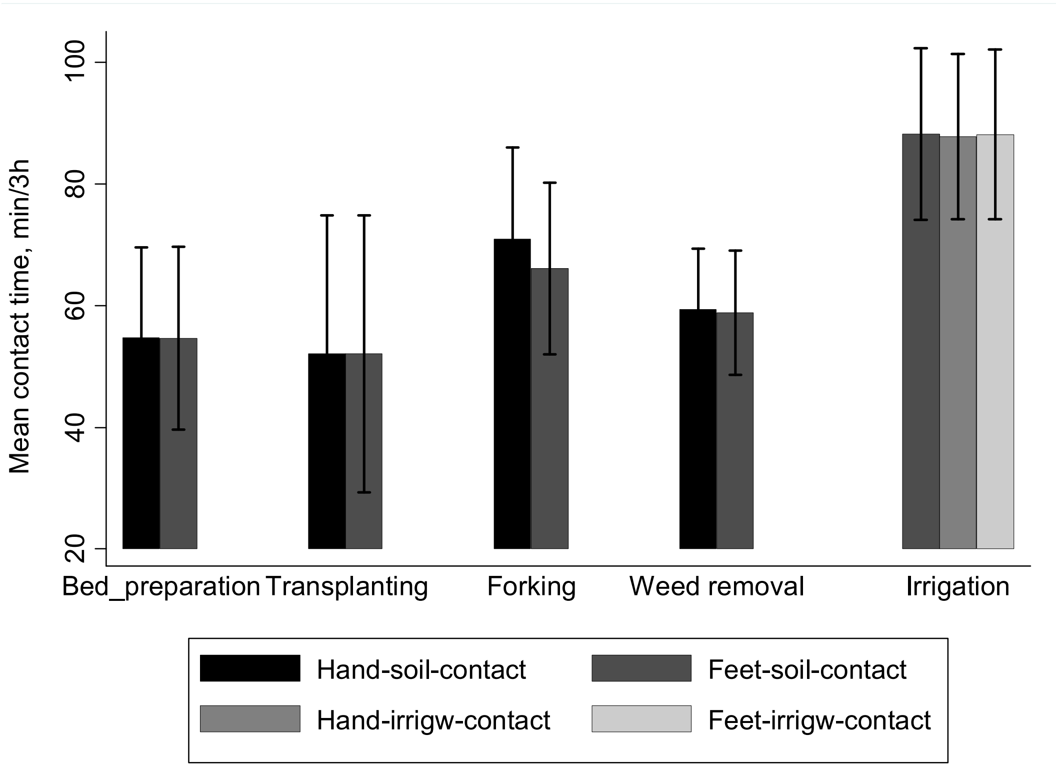


Figure 2.4: Observed farmers contact time (minutes) per 3 hour observation period, by contact type and farm activity.

**Error bars represent 95% CI of the mean.*

2.3.4 QMRA and risk to farmers

From the Poisson distribution, farmers were likely to ingest a minimum of 2 *E. coli*/d and a maximum of 18 *E. coli*/d, if they ingested soil of average quality 2.3 Log *E. coli*/g (Table 2.5). For the maximum soil contamination of 4.1 Log *E. coli*/g, farmers could ingest between 126 *E. coli*/d and 1,259 *E. coli*/d. Farmers were likely to spend an average contact time of 103 d/y and a maximum of 132 d/y in direct contact in the fields for all activities, after assuming 100% feet contact to soil. The median norovirus infection risk for farmers exposed to soil of quality 2.3 Log *E. coli*/g (average contamination) and ingesting between 10 – 100 mg/day soil was therefore estimated to be 8.5 x 10^{-3} pppy and 3.4 x 10^{-3} pppy for exposures of 337 days and 132 days respectively (Table 2.6). When exposed to soil of the highest contamination (worst case scenario) of 4.1 Log *E. coli*/g, the median norovirus infection risk for farmers increased to 0.42 pppy; and 0.19 pppy for an exposure of 337 days and 132 days respectively (Table 2.6).

Table 2.6: Daily dose of *E. coli* ingested by farmers ingesting 10 – 100 mg of soil

Soil quality (Log *E. coli*/g)	**Soil ingested (mg)**	**Dose of *E. coli* ingested**	
		Mean dose	95% Confidence interval
Average soil concentration			
$10^{2.3}$	10	1.8	0, 5
	100	17.8	10, 27
Max. soil concentration			
$10^{4.1}$	10	126	104, 149
	100	1,259	1189, 1327

Table 2.7: Median norovirus infection risks to farmers from the involuntary ingestion of 10 - 100 mg of wastewater-saturated soil per day for 337 and 132 days per year estimated by 10, 000 Monte Carlo (MC) simulations

Soil quality (*E. coli*/g soil)	**®Reported exposure frequency (337 days exposure)**		**Observed exposure frequency (132 days exposure)**	
	*Median norovirus risk pppy	95-percentile norovirus risk pppy	*Median norovirus risk pppy	95-percentile norovirus risk pppy
$10^{4.1\dagger}$	0.42	0.44	0.19	0.21
$10^3 – 10^4$	0.21	0.23	9.3 x 10^{-2}	1.0 x 10^{-1}
$10^2 – 10^3$	2.3 x 10^{-2}	2.5 x 10^{-2}	9.7 x 10^{-3}	1.1 x 10^{-2}
$10^{2.3\ddagger}$	8.5 x 10^{-3}	9.1 x 10^{-3}	3.4 x 10^{-3}	3.7 x 10^{-3}
$10^1 – 10^2$	2.4 x 10^{-3}	2.6 x 10^{-3}	9.7 x 10^{-4}	1.1 x 10^{-3}

*Karavarsamis-Hamilton MC simulations. Assumptions: 0.1-1 norovirus per 10^5 *E. coli*, no pathogen die-off, disease/infection ratio 1:1, † Maximum soil contamination, ‡ Average soil concentration. ®Reported exposure frequency reflects only the days farmers report in the field but does not necessarily reflect the actual time farmers spent in the field, or were engaged in risky activities that expose them to faecal pathogens (observed exposure frequency).

2.4 Discussion

This study found high concentrations of *E. coli* in irrigation water and farm soil. High levels of *E. coli* in soil posed the highest risk as a result of frequent hand to soil, and hand to mouth contacts especially during weeding. On average farmers had 10 events of hand-to-mouth contact per day, and the large majority of farmers were found to work bare feet in their fields for 81% of the time. Based on the WHO developed QMRA models, farm practices in Accra exceeded maximum permissible disease risks.

2.4.1 Irrigation water and farm soil quality

The study found that irrigation water sources used for vegetable cultivation were highly contaminated, with 84% of water samples exceeding the old WHO water quality standard of 3 log *E. coli*/100ml for unrestricted irrigation [27]. The high concentrations of *E. coli* found in irrigation water were similar to those found previously in Ghana [60, 67], though significantly lower than those found in India and Pakistan where farmers were found to use untreated wastewater [30, 41]. Unlike in Pakistan where farmers used raw sewage from a wastewater treatment plant, wastewater used by farmers in Accra was diluted by rainwater, or other sources of storm water. Although water quality was the main factor affecting the presence and concentrations of *E. coli* in soil, the use of poultry manure further contributed to increased levels of *E. coli* in soil. The concentrations of *E. coli* found in soil were lower (2.3 Log *E. coli*/g vs. 3.0 Log faecal coliform/g) than those found previously in Ghana [67]. *E. coli* is an indicator organism for faecal contamination, and the high concentrations found in irrigation water and farm soil, are likely to indicate the presence of a variety of pathogens.

Few studies have enumerated the actual concentrations of pathogens, including viruses in wastewater used for irrigation due to high cost, and poor viral detection efficiencies [115]. Virus concentrations in untreated wastewater have, however, been reported to range from 10^2 to 10^6, though their numbers may depend on region, climate and season [21]. The Norovirus and adenovirus concentrations in this study ranged from 1.0×10^4 to 1.0×10^8 gene copies/100 ml, and were found to be higher (mean, 1.3×10^7 vs. 1.6×10^4, and 4.2×10^5 vs. 6.5×10^4, respectively) than those reported in a recent study in Accra [60]. However, the proportion of irrigation water samples positive for norovirus GII in this study was far lower (15% vs. 80%), while the proportion of positive samples for adenovirus was similar (55% vs. 47%). Seasonality could have played a role, with viruses generally found to survive for longer periods at lower temperatures, and also at higher relative humidity [116, 117]. Virus survival is also enhanced in polluted water due to the protective effect they may receive when adsorbed onto suspended solid material in the dirty water [22, 23]. This could also explain the higher prevalence of adenovirus in wastewater apart from the fact that adenoviruses also have high resistance to ultraviolet (UV) light [22].

2.4.2 Faecal contamination exposure pathways

The WHO QMRA model calculates permissible disease, or infection risk for farmers using wastewater on the accidental ingestion of wastewater contaminated soil during agricultural activities. However there is little evidence to support the assumption that the key risk to farmers is through the soil route, though one study has reported that agriculturalist and archaeologists have higher soil interaction than other workers [118], and that all members of an exposed population will involuntarily ingest at least small quantities of soil adhering to the skin of fingers because of hand-to-mouth activity (Ferguson and Marsh, 1993). This study found the highest concentrations of *E. coli* in irrigation water, and significantly lower concentrations in soil. Farmers, however, spent a higher proportion of their time in contact with soil (> 80%) than with irrigation water (49%). In this study farmers were observed to have direct hand to soil, and also hand to irrigation water contacts, though hand to mouth events were only observed during soil related activities, and not during irrigation. These findings also support the WHO QMRA model approach that is based on the accidental ingestion of soil.

The use of watering cans could possibly prevent, or limit farmers' direct hand to mouth contact of irrigation water since farmers rarely put the watering cans down during irrigation. On the other hand, farmers could ingest some wastewater when engaged in other forms of irrigation application such as, basin, spray or sprinkler irrigation. Farmers' prolonged exposure to wastewater could be more significant when investigating pathogens, or chemical risks that occur via dermal contact, rather than through oral ingestion, especially when exposure to wastewater has been identified as a major risk factor for skin disease, as was the case in Vietnam [119].

In terms of soil ingestion, the study found that farmers were likely to ingest a minimum and a maximum of 2 *E. coli*/d and 1200 *E. coli*/d respectively. This study did not isolate specific strains of *E. coli,* or other pathogens, and therefore is unable to determine whether this exposure could potentially result in an adverse health effect as big differences exist in the infective dose for different *E. coli* strains, though a recent study reported the infective dose of Enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157 to be 10 CFU [120]. Again, the presence of *E. coli* only indicates the presence of faecal pollution and does not necessarily guarantee the presence of pathogens that can cause diseases to humans. It is however acknowledged that the dose ingested in this study could result in adverse health effect since it is higher than the threshold levels of *E. coli* expected in drinking water and most food that can be consumed uncooked.

The contamination of irrigation water by viruses particularly norovirus could also pose health risk to farmers, as farmers are in frequent contact with wastewater contaminated soil. Enteric viruses are also predominantly transmitted via the faecal oral route with untreated wastewater as one of the major sources of infection [121]. In addition, norovirus has been found to be very infectious with fewer than 10 particles able to cause infection [57], apart from the fact that it can withstand wastewater treatment and survive for long periods in aquatic environment [87, 122], and hence its potential to cause adverse health effects to wastewater farmers.

2.4.3 High risk farming activities

All major farm activities were found to expose farmers to faecal contamination, though irrigation, 'forking' and weeding were regarded as the key risk activities. The large amount of time spent by farmers on irrigation comes as a result of the manual method of irrigation application, and the long distances farmers walk to access irrigation water. In this study, farmers spent about 80% of their total working time accessing irrigation water, or irrigating, and was higher than previous estimates (40% vs 70%) in Accra and Kumasi [99, 123]. Only 7% of farmers in this study were seen to wear boots, and often only for short periods, while irrigating, as was shown by studies in other countries (Kenya, Pakistan and Côte d'Ivoire) where between 5% and 19% of farmers reported to wear boots, often citing discomfort, heat and the muddy fields as reasons why they did not wear footwear [70, 71, 104, 105]. In India and Pakistan hookworm infection was found to be the main infection associated with the use of wastewater by farmers, and the lack of use of footwear was cited as one of the main risk factors [41, 104]. In Ghana, stool surveys among wastewater farmers have not been conducted, and as a result it is unknown how irrigation practices, and the lack of footwear affect hookworm prevalence in farmers.

"Forking" and removing weeds were found as the major farming activities driving farmers' risk of accidental ingestion of soil. This was due to the high frequency of hand-to-mouth events associated with these two activities, which are often undertaken simultaneously using hand-held weeding knives and the bare hands. Farmers' hands become contaminated as they try to remove weeds, stones and other waste materials, and this coupled with frequent wiping of sweat from the face due to the heat and the strenuous activities, and the frequent consumption of food, make these high-risk activities. The use of chicken manure was reported to be done by between 60% and 99% of farmers in this study and in other studies by between 70% and 98% of farmers in Ghana [48], with high concentrations of *E. coli,* and the fact that the manure is often applied without the use of protective clothing makes this another key health risk that is not included in the QMRA assessment, and would not only apply to wastewater farmers, but also to regular farmers. Finally, farmers' failure to wash their hands with soap, and consumption of cold food could present another risk pathway for the transmission of faecal pathogens to farmers.

2.4.4 Health risks and the WHO guidelines and policy implications

This study found that the use of wastewater in Accra (and potentially other places with similar farming practices) exceeded the permissible norovirus infection risks (1.4×10^{-3} pppy) corresponding to a DALY loss of 10^{-6} pppy set by the WHO for an exposure of 337 days a year. Similar findings were also found by Mara and Sleigh [124] and Mara *et al*. [59], where wastewater farmers' norovirus infection risk exceeded guideline thresholds by at least one order of magnitude if they ingested 1-10 mg or 10-100 mg of wastewater saturated soil for 100 and 300 days respectively. An earlier study in Accra, Ghana, also found farmers' risk of rotavirus infection (7.6×10^{-2}) to exceed guideline value for rotavirus diarrhoea in developing countries (7.7×10^{-4}) by 2 order of magnitude, after ingesting 10-100 mg of soil for 150 days [66]. The fact that some studies assume a fully-saturated wastewater soil and substitute wastewater quality for soil quality could, however, lead to bias results, as other contaminants have been identified to contribute to soil quality. The results from experimental studies in Italy and Serbia corroborated this argument by concluding that other environmental sources such as wild animals and birds affected the soil quality, rather than irrigation water or factors linked to irrigation practice [125, 126]. The study found that soil samples taken before irrigation harboured higher concentrations of *E. coli* than in soil during irrigation with treated wastewater or channel water, which had low *E. coli* levels.

In the current study, farmers' risk was found to diminish by at least 50% if an actual observed exposure time to water and soil (132 days), or contact in the field was used. Although the use of self-reported time could lead to overestimation of farmers' risk; this influence might be more significant when assessing risk transmitted via dermal contact, and not through oral ingestion. A better approach to estimate farmers' risk due to soil ingestion would, however, be the use of actual hand-to-mouth contact since these events depend more on the type of farm activity performed, and not necessarily on how much time farmers spend in the field. Currently, the design of the WHO QMRA model does not consider farmers risk via skin or dermal contacts (e.g. hookworm infection). For this type of transmission route, the use of the direct observed contact time would be more appropriate since the self-reported time does not necessarily reflect the actual time farmers spent in the field, or were engaged in risky activities that expose them to faecal pathogens. Moreover, further studies, in the form of repeated observations, or longer observations over the course of the year would be required to confirm farmers actual contact time to faecal pathogens, and to better understand their risk behaviours and practices. This is particularly necessary as direct exposure frequency to faecal pathogens in this study was only based on a single 3 h observation per farmer, and also excluded contact to faecal matter during manure application.

The maximum permissible additional disease risk has been under discussion with some arguing that it is too strict for wastewater use in agriculture [112]. This study showed that farmers' occupational risk was within acceptable limits (i.e. within a tolerable norovirus infection risk of 0.14 pppy) for a DALY loss of 10^{-4} pppy but not for the current guideline of DALY loss of 10^{-6} pppy. Only the risk corresponding to the highest soil contamination for an exposure of 337 days exceeded this tolerable risk. A DALY loss of 10^{-4} pppy also means that farmers are likely to contract norovirus infection/disease once every 7 years instead of a zero chance in their life span for a DALY loss of 10^{-6} pppy. The reasons for the use of a relaxed DALY of either 10^{-5} pppy or 10^{-4} pppy was that the resulting norovirus/rotavirus disease risk would still be lower than the actual global incidence of diarrhoeal disease of 0.1 – 1 pppy in both developed and low and middle-income countries [127]. In addition, it would also result in a reduction in the cost required for wastewater treatment; and hence the extra money saved could be used for other risk reduction interventions.

Although high, the estimated risk from this study should be interpreted with caution. First, the risk arising from the mean soil quality was found to safe (i.e. within the acceptable limits), though soil samples (51%) with quality just above the mean (2.4 Log *E. coli*/g) resulted in a risk higher than the guidelines limit. Even with this quality, farmers' risk would be within the acceptable limits, or would be marginally safe if exposure (ingestion) to contaminated soil was at most 300 days (9.0 x 10^{-3}). There were limitations of the model that was used to estimate the risk. The model used published ratios between *E. coli* and norovirus and not necessarily the actual concentrations of norovirus. The use of these ratios often assume a linear relationship between the indicator organism and the pathogen and also ignore other factors which could influence this correlation and hence there are uncertainties in the ratios. There is also inadequate evidence to support the widely used ratio of 1:10^5 *E. coli*/faecal coliform to virus relationship, which was based on a study in northeast Brazil [128]. A recent study in Ghana found an average of one norovirus GII to $10^{3.2}$ *E. coli* or $10^{4.8}$ thermotolerant coliforms, from its quantifiable irrigation water samples, which suggest that the NV-GII to *E. coli* ratio is much larger than the widely used ratio of 1:10^5 [60]. In the current study, the ratio of means between norovirus and *E. coli* was estimated as 2.1 x 10^{-2} or 1:$10^{1.7}$ (1.9 x 10^1 genome copies/mL vs. 8.9 x 10^2 *E. coli*/mL, N=67) from irrigation water samples analysed for both *E. coli* and norovirus, which is also much larger than the common ratios used in recent publications. The other limitation is that the study did not assess for helminths and protozoans and hence the model could underestimate farmers' risk, though the risk associated with viruses is generally considered high enough to adequately protect farmers from bacterial and protozoan infections. The use of soil quality in the risk model instead of water quality as used in some other studies is, however, considered as the "closest" and a better estimate of farmers' risk due to faecal-oral ingestion.

An updated version of the WHO QMRA model should prioritise and incorporate actual hand-to-mouth events in the model since these models often deal with ingestion of contaminated products such as soil, irrigation water and produce. On the other hand, feet contacts should be the focus when dealing with helminth infections (especially hookworms), or other infections transmitted via dermal contact. In terms of pathogen reductions, farmers' risk of 0.42 pppy means that reducing the contamination of irrigation water by two to three log units per 100ml irrigation water or 100g soil (assuming a fully saturated soil) will keep farmers occupational health risk within acceptable levels for a DALY loss of 10^{-6} pppy. A significant part of soil contamination was attributed to the use of chicken manure, and hence adequate treatment of the manure before application is also recommended to reduce farmers' risk; further, manure safety management should form part of the WHO guidelines.

Although these reductions in microbial contamination can be achieved by simple wastewater treatment such as the use of the three-tank or three-pond system which is operated as a sequential batch-fed process [124]; in the short term, wastewater treatment seems an unlikely intervention as, farmers will be unable to invest in wastewater treatment due to land insecurity, and the high costs. Apart from conventional wastewater treatment, Keraita *et al.* [129] have demonstrated that allowing irrigation water in ponds to settle for 6 days could result in a 4 Log unit reduction of thermotolerant coliforms. Again, farmers might find this intervention difficult to adopt due to the long waiting periods and the fact that farmers seem more concerned about keeping their produce fresh for higher yields and profits. Instead, local authorities and other stakeholders should collaborate with farmers by providing credit or loan schemes and also increase land security to farmers who adhere to agreed and prescribed safe practices. This in turn could motivate farmers to invest more in on-farm risk reduction measures such as on-farm sedimentation ponds, and also adopt other good agriculture practices as well as personal and environmental hygienic practices that could reduce both occupational and consumer risk.

2.5 Conclusion

This study found exposure to soil as the critical pathway of pathogen risk in wastewater farmers as a result of hand-to-mouth events, and hence the findings validate the WHO QMRA approach which bases farm risks on the accidental ingestion of soil. Farm practices were also found to exceed the WHO health based target of $\leq 10^{-6}$ DALY loss pppy; though the limitations of the model make the results inconclusive to provide sufficient evidence on the actual risk to wastewater farmers. The study recommends the incorporation of hand-to-mouth soil events in QMRA models, and the use of actual pathogen concentrations in soil and in irrigation water to estimate farmers' risk. It also recommends models for other transmission pathways such as dermal contacts and in that case the use of a much lower exposure frequency for contact with soil (~150 d/y) or wastewater (120 d/y) by agricultural workers, and also a relaxed DALY loss of 10^{-4} pppy.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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SECTION A – Student Details

Student	Prince Antwi-Agyei
Principal Supervisor	Dr Jeroen Ensink
Thesis Title	Wastewater use in urban agriculture: an exposure and risk assessment in Accra

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	My supervisor and I designed the study. I developed all data collection tools and collected field data. I also performed all statistical analysis and was the lead author for the manuscript.

Student Signature: ____________________ **Date:** ______________________

Supervisor Signature: __________________ **Date:** _____________________

Chapter 3: A farm to fork risk assessment for the use of wastewater in agriculture in Accra, Ghana

Photo 3.1: A farmer fetching irrigation water from a municipal wastewater drain in Accra, Ghana

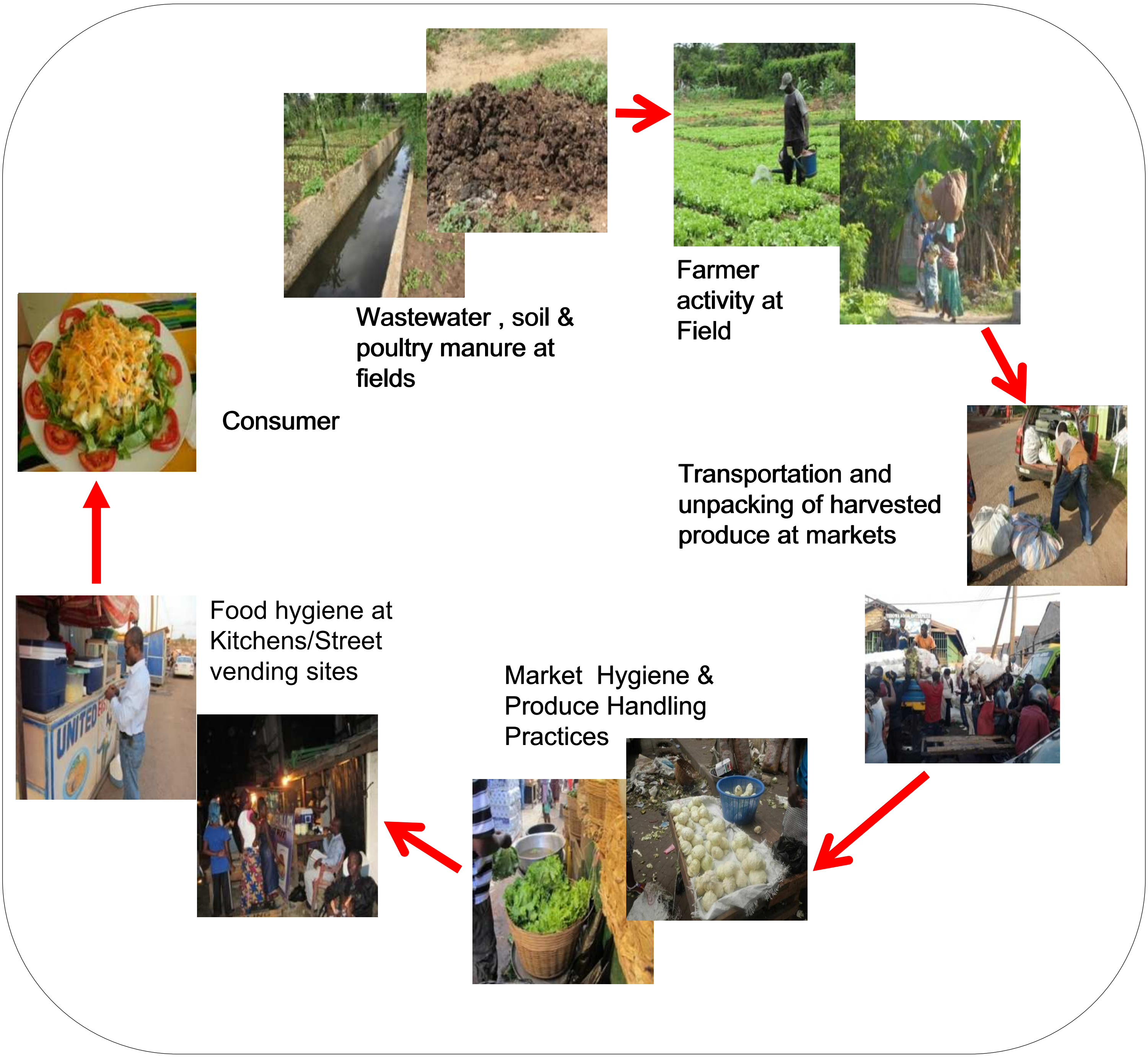

Photo 3.2: A farm to fork assessment of produce quality

Abstract

The need to minimise consumer risk, especially for food that can be consumed uncooked is a continuing public health concern particularly in places where safe sanitation and hygienic practices are absent. The use of wastewater in agriculture has been associated with disease risks though its relative significance in disease transmission remains unclear. This study aimed at identifying key risk factors for produce contamination at different entry points of the food chain.

Over 500 produce and ready-to-eat salad samples were collected from fields, markets, and kitchens during the dry and wet seasons in Accra, Ghana, and over 300 soil and irrigation water samples were collected. All samples were analysed for *E. coli*, human adenovirus and norovirus using standard microbiological procedures, and real time RT-PCR. Finally, critical exposures associated with microbial quality of produce were assessed through observations and interviews.

The study found that over 80% of produce samples were contaminated with *E. coli*, with median concentrations ranging from 0.64 to 3.84 Log *E. coli*/g produce. Prepared salad from street food vendors was found to be the most contaminated (4.23 Log *E. coli*/g), and that consumption of salad exceeded acceptable health limits. Key risk factors identified for produce contamination were irrigation water and soil at the farm level. Storage duration and temperature of produce had a significant influence on the quality of produce sold at markets, while observations revealed that the washed water used to rinse produce before sale was contaminated. The source of produce and operating with a hygiene permit were also found to influence salad microbial quality at kitchens. The results of this study suggest a clear need to manage produce risk factors at all domains along the food chain, though it would be more effective to prioritise at markets and kitchens due to cost, ease of implementation and public health significance.

Keywords: Wastewater use, produce quality, ready-to-eat salad, microbes, risk factors, urban agriculture, Ghana

3.1 Introduction

Although the full extent of disease burden attributable to food-borne diseases is largely unknown, food hygiene and food safety are major public health concerns. In 2005, for example, the World Health Organisation (WHO) attributed 1.8 million diarrhoea-related deaths largely to contaminated food and drinking water [130]. In the United States alone, an estimated 9.4 million episodes of food-borne illness, with 55,961 hospitalizations and 1,351 deaths are recorded every year [131]. Food-borne diseases result not only from consuming food contaminated with pathogens such as bacteria, viruses and parasites, but also chemicals or bio-toxins (WHO, 2011). The most often reported microbial agents related to food-borne diseases are *Salmonella* spp, norovirus, *E. coli Clostridium perfringens* and *Campylobacter* spp [130, 131].

The risk factors for produce contamination are diverse, and may include environmental, animal and human sources. The use of urban wastewater for irrigation, post-harvest practices including market handling, and poor hygienic practices at kitchens have all been linked to produce contamination and disease outbreaks [21]. Although the health risks arising from urban wastewater use in agriculture seem obvious; how consumer risk changes from field, to market, and to household are unclear and poorly documented. In most past studies, there tends to be a focus on disease risks analysis at the farm domain with very little at the market and kitchen domains. This lack of systematic assessment of food hygiene and safety along the complete food chain was the main thrust for the development of the Hazard Analysis and Critical Control Points (HACCP) in the food industry. The HACCP helps identify critical pathways along the food chain where interventions could be prioritised and hence, specific risk-based targets can be developed to control hazards at the different steps in the food production chain [132]. This study adopted a HACCP approach to identify key risk factors associated with the microbial quality of produce and ready-to-eat salad along the food chain.

3.2 Materials and Methods

3.2.1 Study area and site selection

The study was conducted in Accra, the capital city of Ghana with a population of 1.9 million [95]. Seven major agricultural sites were identified where poor quality water was used for the cultivation of salad vegetables, including lettuce, spring onions, cabbage and local vegetables. Most crops were irrigated through the use of watering cans. Farmers sell their produce mainly to market vendors, but also to restaurants and street food vendors. Markets in Accra are classified into five types: central markets, neighbourhood markets, night markets, specialist markets, and privately managed markets [133]. The central markets serve as the largest platforms for vegetable sales, attracting traders from both within and outside Ghana. In Accra, the street food sector constitutes one of the biggest informal categories within the food industry. A popular category of street food vendor in Accra is the “check-check” seller. These are vendors who mostly sell cooked, or fried rice with salad (fast-food). Salads are normally prepared from lettuce, cabbage, carrots or spring onions, and can be mixed with or, without salad cream.

The three largest wastewater irrigated sites in Accra were selected for this study – Korle Bu, Dzorwulu and Marine Drive. Only farmers with at least one bed of ready-to-harvest lettuce at the time of study were included. Farmers were randomly selected using their farm beds/plot as identification. Three central markets (Makola, Agbobloshie and Kaneshie) were also included in the study. Vendors who were thought to sell both cabbage and lettuce were included in the study, and were randomly selected using their market stalls as identification. ‘Check-check’ vendors were recruited from a list of food vendors previously identified by a transect walk in two neighbourhoods (Old Fadama and Alajo) in Accra. Restaurants (including hotels) in Accra where salad was served to the public were also included in the study during the rainy season, and were selected on the basis of their “star” rating, location and popularity from the database of restaurants and hotels from the Ghana Food and Drugs Authority (FDA).

3.2.2 Data Collection

Sample collection and analysis

Lettuce, soil and irrigation water samples were collected from wastewater irrigated fields, while lettuce and cabbage were collected from local markets. Sample collection at farms and markets was done between 7:00 hrs and 10:00 hrs and between 18:00 hrs and 21:00 hrs at street vending sites, all at peak working periods. Ready-to-eat salad samples from restaurants were collected between 10:00 hrs and 15:00 hrs. All samples were collected from September to December 2012 in the dry season and from July to August 2013 in the rainy season. Irrigation water samples were collected from where each farmer was observed collecting water for irrigation, while soil samples were collected within an area of 3 m^2 of where each farm produce sample was collected. The site conditions of where each irrigation water and soil sample was collected were recorded using structured observation sheets (Annexes 1a & 1b).

At farms and markets, farmers and vendors were asked to place produce directly into plastic sampling bags (Whirl-Pak, USA) after they had cut off any roots to prevent unrelated contamination (Annexes 1c & 1d). The temperature (ambient temperature) of produce at markets was taken just before sample collection using a hand held meter (ETI 226-010 ThermaLite, ETI Ltd, UK). For prepared food, vendors were asked to place the food sample into the opened sampling bag using whatever means (e.g. hands, utensils) but a note was made on how the food was handled (Annex 1e). The presence of flies, the distance to open drains, refuse, and defaecation areas were also recorded, while observations were made on how the produce was displayed during sample collection. All collected samples were placed in an ice-box, and transported to the laboratory within 2 hours of collection for immediate processing, or stored in a refrigerator at 4°C until processing. At the laboratory 500 ml of sterile PBS (phosphate buffered saline, pH 7.2) was added to the bags, which were then vigorously shaken, and the surface of each piece of produce gently massaged through the bag before being processed, and analysed for *E. coli,* human adenovirus and norovirus genomes I and II. A 10 g of ready-to-eat salad sample was measured into a sterile tube, vortexed and shaken vigorously at room temperature before the supernatant was processed for the *E. coli*, norovirus and adenovirus assays. For farm soil, 10 g of the sample was measured into a sterile tube and 20 ml of sterile PBS added to it before 10 ml of supernatant was used for the assays.

All samples were processed using the membrane filtration technique with BBL MI agar (Beckton Dickinson, Sparks, USA) to determine the prevalence and concentrations of *E. coli* [109]. Serial dilution ranges were pre-optimized to ensure that ranges allowed enumeration of roughly 95% of samples, per sample type. RNA was extracted using the Qiagen Viral extraction kit (Qiagen, Venlo, Netherlands), and DNA using the MPBio FastDNA kit for Soil (MP Biomedicals, Santa Ana, USA). Virus presence/absence and inhibition in water, soil and produce/prepared salad was determined using Quantifast Pathogen IC Real Time – Polymerase Chain Reaction (RT-PCR) and PCR kits. Norovirus GI and GII and adenovirus concentrations were determined using Qiagen OneStep kits [108].

Observations

Observations were conducted in both seasons using structured observational guide. Each farmer at wastewater irrigated fields and each vendor at markets was observed for one 3 hour session from 7 am – 10 am while each street food vendor was observed from 6 pm – 9 pm. Farmers were observed during their farming activities including method of irrigation, application of poultry manure, and harvesting of produce (Annex 4a). Market and street food vendors were observed on where and how they displayed and stored their produce, and any methods of treating produce/salad (washing, use of disinfectants) (Annexes 4b & 4c). In addition, general sanitation, including refuse, open drains, visible faeces, defecation areas as well as the presence of flies were observed. Participants were told that the observations were aimed at learning more about their general activities at farms, markets and street food vending sites, and not specifically to document critical health risk behaviours.

Questionnaire

At the end of each participant observation, a standardised questionnaire was verbally administered to farmers, market salespersons, street food vendors, as well as chefs in restaurants (Annexes 2a, 2b & 2d). Questionnaires explored the sources, and the methods of displaying produce at markets and ready-to-eat salad at kitchens. It also covered where vendors sold and how produce was stored. At kitchens, the method of treating salad leaves as well as when the salad was prepared were recorded. In addition, the personal characteristics of participants including age, sex, religion, occupation and education were recorded.

3.2.3 Sample size

Sample sizes for produce were determined based on 80% power and 5% significance level to detect a 5% to 10% difference in faecal coliform concentration levels between produce at farms and markets [48]. This resulted in a sample size of 80 produce samples each from farms and markets during each of the dry and wet seasons. Similarly, sample size for consumers of salad produce was determined to detect a 20% difference in increased awareness or knowledge of health risk associated wastewater irrigation between salad consumers and non-consumers based on a similar study in Ghana [134]. This also resulted in 160 each of street food consumers and buyers of salad produce at markets (domestic consumers). The number of soil and irrigation water samples collected at farms corresponded to the produce samples collected at farms during each season. Fifty samples of ready-to-eat salad were collected from 30 fast-food sellers and 20 chefs from 20 hotels and restaurants.

3.2.4 Data Analysis

All data were analysed using STATA 12 (StataCorp LP, College Station, USA). All samples with undetectable concentrations were multiplied by 0.5, the lower limit of detection for *E. coli* dilutions, or per standard curve in molecular virology analysis. Distributions of *E. coli* concentrations in environmental and food samples were tested for normality using the Shapiro-Wilk test, and inverse normal plots. Concentrations were log transformed for calculations of means, standard deviations, and 95% confidence intervals (CI). The Mann-Whitney test was used to test for the difference in median concentrations of *E. coli* of street vended salad and irrigation water between seasons while the Kruskal-Wallis test was used to compare the median concentrations of produce samples among the different domains. Apart from street vended salad, two sample t-tests were used to compare the mean *E. coli* concentrations of produce at farms, markets and restaurants between the dry and rainy seasons as their distributions were normal after log transformation. Linear and logistic multiple regression models were used to assess risk factors for produce quality. Using a forward stepwise-regression approach, only risk factors that were significantly associated with produce quality at 20% were included in the multiple regression model [135]. Statistically significant associations in the multivariable analysis were measured at 5% significance level using likelihood ratio test. Multicollinearity was assessed using the variance inflation factor [136].

Microbial concentrations in irrigation water were reclassified as ≤ 3 Log *E. coli*/100ml and > 3 Log *E. coli*/100ml following the old WHO water quality standard set for wastewater use [27]. Concentrations in salad produce were also regrouped as ≤ 3 Log *E. coli*/g and > 3 Log *E. coli*/g [137] or ≤ 2 Log *E. coli*/g and > 2 Log *E. coli*/g which define guidelines limits considered as microbiologically satisfactory for consumption, or not [138, 139], and the proportion of produce and prepared salad with *E. coli* concentrations that meet these guideline limits were then noted.

Quantitative Microbial Risk Assessment (QMRA)

In order to determine whether the consumption of wastewater irrigated produce met health standards a quantitative microbial risk assessment (QMRA) model developed for the WHO guidelines for safe use of wastewater in agriculture was used [102]. The model uses the Karavarsamis-Hamilton method [111], together with the norovirus dose-response model by Teunis *et al*. [57]. A maximum tolerable additional disease burden of 10^{-6} disability-adjusted life year (DALY) loss per person per year (pppy) as used in the WHO guidelines was adopted, which equates to a maximum permissible norovirus (NV) infection risk of 1.4 x 10^{-3} pppy. Similarly, the tolerable NV infection risks corresponding to a DALY loss of 10^{-4} pppy, as proposed by those in favour of a more relaxed DALY loss, was 0.14 pppy [112]. The frequency and quantity of salad consumption were determined from questionnaire-based consumer surveys and laboratory experiments (Chapter 4). The amount of salad consumed on a daily basis at home was estimated using the average weight of lettuce bought at markets, and the number of lettuce used to prepare a salad meal for a family of 4 (Chapter 4). The quantity of salad consumed at street food vendor level was based on a national consumer survey by the International Water Management Institute in Ghana [94]. Five different consumption exposure or pathway models were used to estimate the dose of norovirus ingested and subsequently the risk of infection. The water model used the quality of irrigation water to estimate pathogen dose ingested based on the amount of wastewater left on produce after irrigation. All other models (Farm produce model, market produce model, restaurant salad model and street food model) used direct *E. coli* concentrations on produce, or in prepared salad to estimate the dose ingested. A maximum pathogen reduction of 2 Log units arising from produce washing or disinfection was assumed for farm and market produce models; while no pathogen reduction was assumed for prepared salad models [140].

3.2.5 Ethical Considerations

Ethical approval was received from the London School of Hygiene and Tropical Medicine (reference number - 6236) and from the Noguchi Memorial Institute of Medical Research, Accra, Ghana (Reference number – DF22). The study was explained to and agreed by local leaders, and written informed consent was obtained from each individual study participant.

3.3 Results

3.3.1 Microbiological quality of produce

A total of 422 produce samples were collected, 159 from wastewater irrigated fields and 263 from markets (134 lettuce & 129 cabbages), and a further 79 samples of ready-to-eat salads; 59 from street food vendors and 20 from restaurants. Ready-to-eat salad from street food vendors was found to be the most contaminated with 98% of all collected samples positive for *E. coli*, followed by market lettuce (97%), farm lettuce, (96%), market cabbage, (89%) and restaurants salads (80%). Overall, street salad was found to have the highest concentrations of *E. coli* (4.1 Log *E. coli*/g) among all produce and prepared salad from the other domains (Figure 3.1). Farm lettuce was found to contain significantly higher levels of contamination than market lettuce for the combined season (3.3 vs 2.9 Log *E. coli*/g, $p < 0.001$). The concentrations of *E. coli* on farm lettuce during the dry season were found to be higher than during the rainy season ($p < 0.001$, Table 3.1), in contrast to lettuce and cabbage at markets which were found to contain higher concentrations during the rainy season (Table 3.2). None of the produce samples from farms and markets were found to be positive for norovirus GI and GII, while 9% (N = 57) of farm produce, and 7% (N = 85) of market produce samples were positive for adenovirus. Mean concentrations of adenovirus in farm and market produce that tested positive for the virus were 8.1 x 10^3 and 1.9 x 10^4 gene copies/produce, respectively. No street vended salad sample was found positive for either one of the viruses.

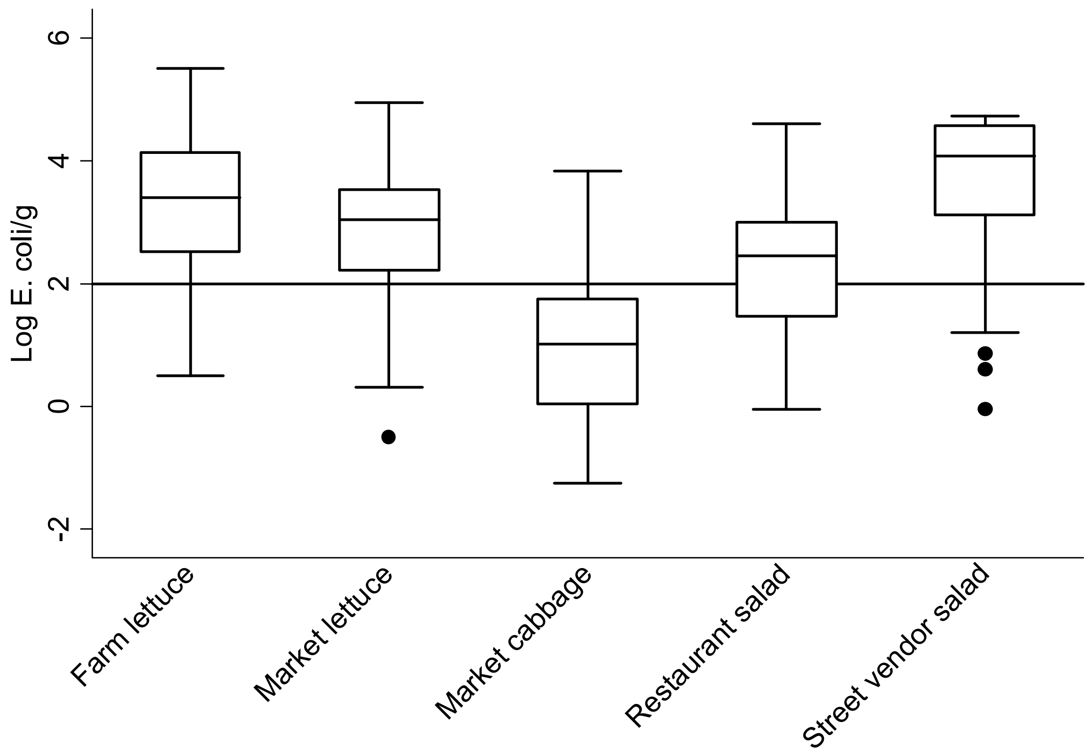

Figure 3.1: *E. coli* concentrations in raw produce and ready-to-eat salad at different entry points along the food chain

Solid horizontal line: limit of E. coli concentration classified as microbiologically satisfactory for consumption (% exceeding 2 Log E. coli/g - street vended salad, 90% (N=59), farm lettuce, 88% (N=159), market lettuce, 80% (N=134), restaurants salad, 60% (N=20), market cabbage, 18% (N=129)).
p-value calculated using Kruskal-Wallis test

Table 3.1: Univariable analysis of risk factors for *E. coli* contamination of produce (lettuce) at farms

Exposure	N	Mean (Log *E. coli*/g)	95% CI*	P – value**
Proximity to open drain				
≤ 3m	7	3.55	2.04, 5.05	0.12
> 3m	73	2.79	2.51, 3.07	
Irrigation water proximity to trash/refuse				
≤ 3m	59	2.92	2.57, 3.28	0.43
> 3m	21	2.67	2.29, 3.06	
Source of irrigation water				
Drain water	36	3.48	3.13, 3.83	< 0.001
Dug-out/pond	41	2.40	2.03, 2.77	
Piped water	3	1.61	-0.63, 3.84	
Irrigation water quality				
≤ 3.0 Log *E. coli*/100ml	24	2.52	1.98, 3.07	< 0.001
> 3.0 Log *E. coli*/100ml	130	3.46	3.27, 3.65	
When produce last irrigated				
≤ 2 days	45	2.97	2.66, 3.28	0.35
> 2 days	35	2.71	2.20, 3.22	
Soil with manure				
Yes	48	3.01	2.70, 3.32	0.19
No	32	2.63	2.11, 3.16	
Soil quality				
≤ 2.3 Log *E. coli*/g	81	3.00	2.72, 3.29	< 0.001
> 2.3 Log *E. coli*/g	76	3.64	3.41, 3.85	
Season				
Dry season	79	3.76	3.57, 3.95	< 0.001
Rainy season	80	2.86	2.58, 3.13	

**95% CI = 95% confidence interval.*
***p-value calculated using t-test or ANOVA*

Table 3.2: Univariable analysis of risk factors for *E. coli* contamination of salad produce at markets

	Lettuce				Cabbage			
Exposure	**N**	**Mean (Log *E. coli*/g)**	**95% CI***	**P – value**	**N**	**Mean (Log *E. coli*/g)**	**95% CI**	**P – value****
Season								
Dry season	54	2.53	2.27, 2.78	< 0.001	49	0.75	0.40, 1.10	0.06
Rainy season	80	3.12	2.92, 3.31		80	1.15	0.89, 1.40	
Type of market								
Main market (under roofing)	44	3.02	2.75, 3.28	0.28	36	1.07	0.68, 1.47	0.60
Open-air/street market	36	3.23	2.93, 3.54		44	1.21	0.86, 1.56	
Display of produce								
On ground (using mats)	15	3.21	2.75, 3.67	0.59	19	1.06	2.75, 3.67	0.92
> 1m above ground	41	3.02	2.73, 3.30		34	1.15	2.73, 3.30	
< 1m above ground	24	3.23	2.84, 3.61		27	1.20	2.84, 3.61	
Vending site concreted								
Yes	69	3.12	2.90, 3.34	0.87	71	1.20	0.92, 1.48	0.24
No	11	3.08	2.55, 3.59		9	0.72	0.11, 1.32	
Produce exposed to sunlight								
Yes	13	3.14	2.68, 3.61	0.90	21	1.16	0.56, 1.75	0.97
No	67	3.11	2.89, 3.33		59	1.15	0.86, 1.43	
Produce covered or not								
Yes	9	2.70	2.02, 3.37	0.13	4	1.33	0.43, 2.23	0.75
No	71	3.17	2.96, 3.38		76	1.14	0.87, 1.41	
Produce storage temperature								
≤ 25 ºC	23	3.24	2.83, 3.64	0.45	31	1.52	1.15, 1.90	0.02
> 25 ºC	57	3.01	2.83, 3.30		49	0.91	0.57, 1.25	
Produce storage time/hr	80	0.028	0.0088, 0.048	0.05	80	0.0021	-0.009, 0.013	0.69

*SD^0 = standard deviation. *95% CI = 95% confidence interval.*
***p-value calculated using t-test or ANOVA*

3.3.2 Key exposures and practices associated with produce quality

The use of poultry manure as soil fertilizer was common and was higher in the dry season than in the rainy season (99% vs. 60%). The study also found that open defecation was common among farmers (73%), although the practice normally occurred away from the main farming areas. Although 68% of market vendors reportedly washed their vegetables (lettuce and carrots) before sales, observation of vendors' washing practices at markets showed that washed water for produce was used without changing it for an average of 22 minutes and the washed water was always contaminated. At markets, at least 80% of harvested produce from farms were mostly sold within 24 hours, but in some cases could be stored for 48 hours for lettuce, and 84 hours for cabbage before sale. At the street food sites, vendors used either public toilets (73%), or market toilets (27%). Generally, environmental sanitation at most street food sites was poor with 87% of the sites without concrete or cement floors (Table 3.3). The 3 hour observations revealed that 33% of street food vendors had their salads uncovered at the time of sampling, and that salad could remain uncovered for an average time close to 100 minutes. Four of the six vendors who were observed to prepare salad at their vending sites, did not wash their hands before salad preparation. At vending sites, produce could be stored for an average time of 10 hours before being used or sold.

3.3.3 Risk factors for produce microbial quality

The concentrations of *E. coli* found on farm produce increased with increased levels of *E. coli* found in soil or irrigation water (Figure 3.2). Seasonality modified the association between farm soil and farm produce quality with lower concentrations of *E. coli* found in the dry season as compared to the rainy season, with a 0.05 Log *E. coli*/g and 0.70 Log *E. coli*/g increase in produce contamination found per unit (Log *E. coli*) increase in soil contamination for the dry and rainy season respectively. In contrast, the effect of irrigation water quality on produce quality was found to be higher in the dry season as compared to the rainy season with a 0.20 Log *E. coli*/g and 0.06 Log *E. coli*/g increase in produce contamination per unit (Log *E. coli*) increase of *E. coli* in irrigation water. The modification by season for the association between irrigation water and farm produce quality was however found to be non-significant ($p = 0.19$). The time of application of irrigation water or poultry manure before sampling was found not to play any significant role on the concentration of *E. coli* found on farm produce (Table 3.1).

Table 3.3: Risk factors for *E. coli* contamination of ready-to-eat salad at street vending sites

Exposure	N	Median (Log *E. coli*/g)	IQR	P – value
Season				
Dry season	29	4.23	3.60, 4.60	0.06
Rainy season	30	3.93	3.13, 4.57	
Proximity to open drain or refuse (n = 30)				
< 3m	23	3.95	2.98, 4.02	0.68
> 3m	7	3.13	2.42, 4.19	
Covered at time of sampling (n = 30)				
Yes	20	4.03	2.83, 4.32	0.61
No	10	3.63	2.92, 4.28	
Vending site concreted (n = 30)				
Yes	4	3.47	3.02, 3.87	0.39
No	26	4.05	2.76, 4.32	
Placement of salad in bag (n = 30)				
Plastic bag	3	4.32	4.31, 4.32	0.14
Spatula/spoon	21	3.37	2.76, 4.25	
Hands	6	4.12	2.92, 4.60	
Salad treatment method (n = 30)				
Salty water	17	3.82	3.11, 4.32	0.15
Vinegar	5	3.91	2.76, 4.15	
Salty water & vinegar	2	1.09	0.79, 1.38	
Water only	6	4.26	3.95, 4.32	
Where produce stored (n = 24)				
At home	3	4.58	4.21, 4.60	0.75
Vending site	11	4.01	3.60, 4.60	
Use immediately	10	4.31	2.62, 4.60	
How produce stored (n = 24)				
On a mat laid on ground	6	4.24	3.58, 4.60	0.26
In a box or container	11	4.60	3.60, 4.60	
Other	7	3.83	1.20, 4.38	
Where salad often prepared (n = 24)				
At home	5	4.58	4.23, 4.60	0.47
Vending site	19	4.08	3.58, 4.60	

**IQR = interquartile range*

***p-value calculated using Mann-Whitney test or Kruskal-Wallis test*

Produce washing practices at markets

Risky practice of displaying produce at markets

Salad preparation and consumption at markets

Street food vending site showing uncovered salad and chopping board used for multiple purposes

Photo 3.3: Exposures and practices at markets and street food vending sites

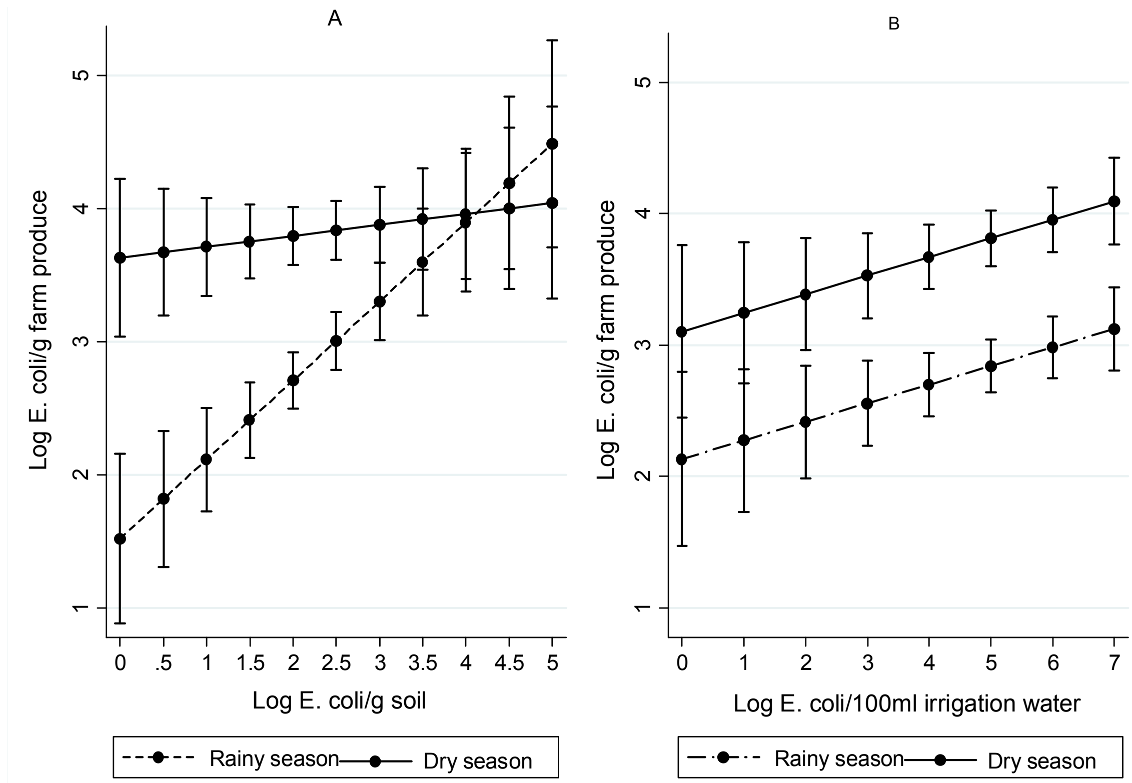

Figure 3.2: Effect of soil (A) and irrigation water (B) on farm produce quality after adjusting for seasonality

Seasonality and soil interaction p = 0.004, 95% CI =-0.85, -0.17. Soil effect on produce contamination (point estimate for unit increase = 0.60 Log E. coli/g produce, 95% CI = 0.32, 0.87, p < 0.001). Effect of irrigation water on produce contamination (point estimate for unit increase = 0.14 Log E. coli/g produce, 95% CI = 0.02, 0.27, *p = 0.027). Error bars = 95% Confidence Intervals (CI).*

No environmental exposures variables were identified at the markets that influenced the concentrations of *E. coli* found on market produce. Only few produce samples were displayed within 3 m of open drains, or within 30 m of a latrine (1.2% and 2.5% respectively, N = 80). However, the storage time of lettuce was associated with significantly (p = 0.05) increased levels of *E. coli* on produce. On average lettuce was stored for 10 hours at markets but for a maximum of 48 hours (2 days) before sales. During the rainy season a 1°C increase in the storage temperature of cabbage resulted in a reduction of 0.28 Log *E. coli*/g.

The method reported to decontaminate salad had a non-significant impact on the quality of street salad, though a combination of salty water and vinegar was found to have the lowest levels of *E. coli* (Table 3.3). At hotels and restaurants, those who operated with a valid hygiene permit had on average 1.53 Log *E. coli*/g less contamination of their prepared salad than those who had no valid hygiene permit (Table 3.4). The source of raw produce was also associated with a borderline significant difference in the average *E. coli* levels in salad sold at restaurants (p = 0.06).

Table 3.4: Risk factors for *E. coli* contamination of ready-to-eat salad at restaurants

Exposure	**N=20**	**Mean (Log *E. coli*/g)**	**95% CI**	**P_1 – value**
Univariable Analysis				
Kitchen type				
Hotel	10	1.79	0.98, 2.59	0.22
Restaurant	10	2.56	1.43, 3.70	
Covered or not				
Covered	11	2.17	1.11, 3.23	0.99
Not covered	9	2.17	1.23, 3.12	
Hygiene Permit				
Yes	17	1.94	1.23, 2.65	0.08
No	3	3.48	2.43, 4.54	
When prepared				
Freshly prepared	11	1.88	0.92, 2.85	0.32
Already prepared	9	2.53	1.50, 3.55	
Source of produce				
Farm gate	4	3.24	1.18, 5.31	0.06
Wholesale market	7	1.14	-0.03, 2.32	
3rd-party supplier	7	2.39	1.27, 3.51	
Supermarket	2	2.88	-1.02, 6.79	
Placing of salad into sampling bag				
Spatula	13	2.00	1.10, 2.90	0.76
Hands	4	2.41	-0.38, 5.21	
Hands with gloves/plastic bag	3	2.61	1.95, 3.27	
Salad treatment				
Vinegar	3	1.77	-2.15, 5.70	0.88
Salt water & vinegar	6	1.95	0.002, 3.90	
Water only	3	2.25	0.23, 4.28	
Others	8	2.46	1.37, 3.55	
Storage time/hr	20	-0.030	-0.05, -0.0043	0.02
Multivariable Analysis*				
Exposure	**Obs**	**Change in mean (Log *E. coli*/g)**	**95% CI**	**P_2 – value**
Hygiene Permit	20	1.53	-0.03, 3.10	0.05
Storage time/hr	20	-0.015	-0.04, 0.011	0.24
Source of produce (farm gate as baseline)	20			
Wholesale market	7	-2.08	-3.56, -0.60	0.01
3rd-party supplier	7	-0.90	-2.34, 0.55	0.20
Supermarket	2	-1.45	-3.52, 0.61	0.15

P_1, *p-value calculated using t-test and Anova*

P_2, *p-value calculated using likelihood ratio test.*

**After controlling for hygiene permit, source of produce and storage time of produce*

3.3.4 Produce quality and infection risk associated with Salad consumption

On average, consumers of street food consume 13 g of salad 4 times/week while domestic consumers consumed 51 g of salad 2 times/week. The annual median norovirus infection risk for the consumption of 10 – 51 g of lettuce salad for 2 – 4 days per week varied across the different exposure models and ranged between 2.6 x 10^{-3} and 0.32 pppy (per person per year), and was highest with the street salad model (Table 3.5). The estimated infection risks from the water model, restaurant and street salad models were all higher than the maximum tolerable norovirus infection risks; while the risk arising from the maximum contamination of the farm and market produce models were also found to be higher than acceptable limits. Only the risks from the consumption of produce of average contamination levels at farms and markets were marginally within the acceptable norovirus infection risks.

Table 3.5: Median norovirus infection risks from the consumption of 10 – 51 g of wastewater irrigated lettuce on 2 - 4 days per week estimated by 10,000 Karavarsamis-Hamilton MC simulations

E. coli **contamination**	**Median norovirus infection risk (pppy)**	**95-percentile norovirus infection risk (pppy)**
Water model (*E. coli*/100 ml)		
3.63 x $10^{5\dagger}$	0.142	0.198
1.48 x $10^{7\ddagger}$	0.997	1.0
Produce/salad quality (*E. coli*/100 g)		
Farm produce model		
2.04 x $10^{5\dagger}$	6.8 x 10^{-3}	9.9 x 10^{-3}
3.16 x $10^{7\ddagger}$	0.650	0.785
Market produce model		
7.59 x $10^{4\dagger}$	2.6 x 10^{-3}	3.7 x 10^{-3}
8.91 x $10^{6\ddagger}$	0.258	0.354
Restaurant salad model		
1.48 x $10^{4\dagger}$	1.3 x 10^{-2}	1.9 x 10^{-2}
3.98 x $10^{6\ddagger}$	0.964	0.992
Street salad model		
4.57 x $10^{5\dagger}$	0.323	0.430
5.37 x $10^{6\ddagger}$	0.988	0.998

Assumptions: 0.1-1 norovirus per 10^5 *E. coli*, disease/infection ratio 1:1.
† Median irrigation water contamination or mean produce/salad contamination
‡Maximum *E. coli* contamination
*No pathogen reduction for prepared salad at restaurants and street kitchens

3.4 Discussion

The results of the study showed that salad produce was faecally contaminated at all entry points of the food chain, with street salad being the most contaminated. Key risk factors identified included farm soil contamination, and the use of wastewater for irrigation. Others were poor hygiene and environmental sanitation, inadequate protection of prepared salad, produce storage time and temperature, and operating with a hygiene permit. Based on the WHO QMRA model consumption of salads in Accra did not meet health standards.

3.4.1 Produce quality from farm to fork

This study found street food salad to be the most faecally contaminated food, though concentrations of *E. coli* found at all sample points were high. The concentrations of faecal pathogens found on lettuce at farms in this study (3.31 Log *E. coli*/g) were lower than those found in previous studies in Ghana, ranging from 5.0 Log MPN/g to 9.0 Log MPN/g [64, 141], though most studies reported contamination in concentrations of total thermotolerant coliform (TTC), which is a much larger group of faecal bacteria, and as a result less specific than *E. coli* as an indicator of faecal pollution [75]. This difference in lettuce contamination levels could also be due to differences in the microbial quality of produce risk factors such as soil, and irrigation water, or the frequency of manure application and irrigation. The *E. coli* concentrations found on lettuce collected from the field in Ghana were however significantly higher, than those in Pakistan (>2,000 *E. coli*/g vs 1.9 *E. coli*/g produce), even though the irrigation water quality was found to be much better in Ghana (9.8 x 10^4 vs 1.8 x 10^7 *E. coli*/100ml). This could most likely be explained by the type of irrigation water application, watering cans in this study, as compared to basin or furrow irrigation techniques which minimizes contact with wastewater; while the much higher temperatures and lower humidity in Pakistan could have promoted much more rapid die-off of *E. coli* on produce [65].

The microbial quality of produce is influenced by a variety of factors which include: the type of vegetable, environmental conditions like temperature, humidity and exposure to sunlight, the type and the application of irrigation water and post-harvest handling. Studies have shown that leafy vegetables with irregular surfaces, more binding sites or several indentations or natural irregularities on their intact surface tend to be more contaminated than smooth surface vegetables like cabbages [142, 143], and this could explain the higher levels of *E. coli* on market lettuce than cabbage in this study. A similar trend was also found by Amoah [141] in three cities (Accra, Kumasi and Tamale) in Ghana where lettuce was found to harbour higher levels of faecal coliform than in cabbage (geometric mean: 1.1 x 10^7/g vs. 3.3 x 10^6/g).

The contamination of street food salad in this study could be associated with poor sanitation (e.g. dust, refuse) and hygiene practices (e.g. uncovered salad, use of chopping board for multiple purposes) at the vending sites. In Kumasi, Ghana, for example, a study found higher levels of thermotolerant coliform (TTC) of 6.2 Log units/100 g in salad from vendors with dirty sites and poor food handling practices, 1.8 Log units/100 g higher than other vending sites within the same location [134]. The cut, or sliced nature of the prepared salad also facilitated the growth of microorganisms, or increased their persistence unlike raw produce which are intact [144]. The concentrations of *E. coli* in street vended salad in this study (3.7 Log *E. coli*/g), although high, were lower than levels found in earlier studies in Accra which were found to range between 5.1 and 6.4 Log cfu/g [145-149], though most of these studies measured faecal coliforms. The higher levels of faecal contamination in those studies could be attributed to the fact that the salad were mixed with salad cream containing egg yolks which has shown to be a good medium for microbial growth [144, 145]. Salad samples collected for this study were without salad cream. The higher contamination of street food salad than salad from restaurants also confirms a study in Kumasi which found higher contamination of TTC in salad sold by street vendors than those sold at cafeterias (5.4 Log cfu/100 g vs 3.8 Log cfu/100 g) [134]. This could also be due to the poor sanitation conditions at street vending sites compared to restaurants or cafeterias.

The higher contamination of produce at farms than at markets emphasises the debate on the relative importance of post-harvest effect including poor sanitation and market handling on the quality of produce. Results from this study agree with earlier findings from Amoah *et al*. [67] in a study in Accra, but contrast with the findings in Pakistan that suggested produce at a local market had at least seven times higher levels of contamination than produce from farm gates [65]. However this study seems to suggest that the differences might partly be influenced by seasonality. The design of the current study did not permit a direct correlation of farm level contamination to produce contamination at markets since produce at farms were not followed to markets to monitor the contamination levels. The geometric mean levels of *E. coli* found on market lettuce in this study (339 *E. coli*/g and 1,318 *E. coli*/g) were 20 times higher than levels found from at markets in Faisalabad, Pakistan (14.3 *E. coli* /g) [65]. The contamination at Faisalabad was attributed to unsanitary market conditions and handling practices including the method of washing produce.

The prevalence of human adenovirus (HAV) on farm produce was the first time viruses have been isolated in farm lettuce at the study sites. A recent study at the same sites analysed for human adenovirus and norovirus in wastewater but not on farm produce [60]. Apart from Norovirus, HAV is a known cause for food-borne infections, and its presence on farm produce could be attributed to the direct use of wastewater and animal manure for vegetable cultivation [89]. Moreover, viruses can survive on harvested produce and can remain infectious for several days up to a period of 5 weeks even during storage [150, 151], and therefore could pose some public health concerns. The study did not analyse samples for helminths though earlier studies in Ghana have found helminth eggs on farm and market produce, and also in prepared salad sold at street food kitchens and also at cafeterias [48, 134].

3.4.2 Health risks associated with produce quality

There is an increasing debate on the permissible level of microbial concentrations in ready-to-eat food. While the International Commission on Microbiological Specifications for Foods (ICMSF) recommends a limit of 1,000 *E. coli*/g produce, the United Kingdom has a threshold of < 20 *E. coli*/g (satisfactory) and ≥ 100 *E. coli*/g as unsatisfactory for ready-to-eat food (including fresh vegetables and mixed salad vegetables) at the point of sale, a standard also adopted by Canada, Australia, New Zeeland and Hong Kong [139]. In Ghana, the national microbiological reference value for ready-to-eat foods including salad is < 100 cfu/g, and is based on the 3-class attribute sampling plan (satisfactory, acceptable, and unsatisfactory) [138]. This is the minimum count of organisms per gram, or per ml below which there would be no risk associated with safety of a food, or the maximum value beyond which a lot would be rejected. Based on the Ghana standards only 11% and 20% of the lettuce collected from the wastewater fields, and local markets could be deemed safe, and 40% and 10% from restaurants and street food vendors respectively. From the results of the QMRA model developed by the WHO for the safe use of wastewater in agriculture, the use of wastewater, and post handling practices at markets and kitchens in Accra are unsafe since the estimated pathogen risks were higher than the recommended level of 1.4×10^{-3} pppy for norovirus. However, for a relaxed DALY loss of 10^{-4}, as proposed by Mara [112], almost all the median annual norovirus risk arising from the use of average produce/salad contamination were within the norovirus acceptable limits (0.14 pppy), though all the worst case scenarios exceeded this limit. Although all exposure models in this study were approximations, the restaurant and street salad models represented the closest estimate of the risk to consumers since these models used fewer assumptions, and are also at the points of direct consumption. The water model is the least reliable, and should not be used in instances where direct concentrations of pathogens on produce or in prepared salad are available. The main disadvantage of the water model is that it assumes that the use of wastewater is the only source of produce contamination, and does not account for other sources of contamination at farms, markets and kitchens. Only the risks from the farm and market produce models were marginally within the acceptable risks, and this could be due to the inclusion of pathogen reduction measures prior to consumption in those models. On the other hand, the risks from these models still exceeded the threshold limits if the worse-case scenarios (use of the highest level of produce contamination) were considered. Although all models were based on an indicator-pathogen ratios, the high risks from these models present a potential public health concern for consumers of salad.

The presence of *E. coli* is an indicator of faecal pollution, but not necessarily a good indicator of disease risk, though signals the possible presence of other pathogens, and hence the need to intervene appropriately since “absolute zero risk” does not exist when assessing microbial risk in food [152]. In order to protect consumer health, a combination of produce washing and disinfection, which have been shown to reduce up to 3-Log unit of pathogens including norovirus is recommended; together with good agricultural practices [63, 140, 153]. Wastewater treatment and crop restriction are key risk reduction measures, but are rarely implemented in low and middle income countries.

3.4.3 Risk factors, health protective measures and policy implications

Probably the most significant public health concern found in this study were the high levels of faecal contamination found in street food salads. This is particularly worrying because up to 800,000 people per day have been estimated to consume this food and other salad related foods from food establishments in major cities in Ghana [94]. The results of the study did not identify specific risk factors for street vended salad, though the use of some cleaning methods seemed to have a protective effect. The time between salad preparation and consumption could be a potential risk factor, as studies have shown that though most sanitizing solutions are capable of reducing microbial concentrations following washing, epiphytic microorganisms can grow rapidly, reaching similar levels as before washing [154, 155]. A key recommendation for street food vendors, therefore will be to prepare salad in small quantities based on customer inflow, in order to prevent contamination due to inadequate storage and inappropriate temperatures. Another recommendation would be to prevent the use of leftover salad mixing with freshly prepared salad which could be another source of cross contamination [145]. Salads sold at hotels and restaurants must be prepared upon customer request, or be refrigerated (below 5ºC) until ready to be served. Generally, microbial growth is slowed down, or stopped at temperatures below 5°C or above 60°C, even though some psychotrophic microbes (e.g. *Listeria monocytogenes*, *E. coli*) may still develop, or multiply below 5°C if storage time is too long [156-158].

The potential effect of farm soil, and irrigation water on salad contamination could not be assessed due to the limitation of the study design. Observations at farm sites showed that some street food vendors bought vegetables directly from farms, and sometimes washed their produce with irrigation water (dug-out), a practice that has also been reported among other market vendors in other parts of Ghana [141]. Other possible sources of salad contamination could be the chopping board, cutting knives and working surfaces used for food preparation at kitchens especially if these devices were used for multiple purposes such as cutting of meat [159, 160]. Street food vendors' practice of not covering salad properly in receptacles during sales could worsen the microbial load due to cross-contamination [144]. This study found lower levels of contamination when a hygiene certificate was in place suggesting that local authorities should require vendors to obtain one. In order for these to work frequent hygiene inspection and monitoring of food premises by food hygiene authorities should remain a key priority. This is particularly necessary since vendors' knowledge, awareness and attitudes on hygiene alone do not necessarily translate into good hygienic practices [161]. Aside from cooking of vegetables and thorough washing and disinfection, domestic consumers can also remove the outer parts of vegetables before salad preparation to reduce the potential risk of pathogens since most pathogenic contaminations are exogenic [162].

Produce quality at market showed non-significant associations with hygiene and sanitation practices, which could be as a result of this study to measure other potential risk factors for produce quality at the markets. Irrespective of these limitations, two possible reasons could account for produce contamination at markets. At first, washing of produce, could introduce microbial contaminants if wash water was contaminated as was shown at markets in Portugal

[163] and Pakistan [65]. A study in Bangladesh, found wash water used for fresh vegetables and fruits to have enteric bacteria concentrations ranging from 1.3 10^3 to 2.0 10^7 cfu/ml [164]. Vendors are advised to wash produce under running potable water, or use multiple batches of potable water in order to prevent produce recontamination, though this requires involvement of local authorities as many markets lack access to clean water. A second reason for produce contamination at market could be environmental contaminants, as observations showed that 90% of vending sites were made of concrete, and therefore reducing the risk of dust as a potential source of produce contamination [165]. This study could not assess the role of factors such as transportation practices along the distribution chain and handling during storage which could contribute to produce contamination at markets.

At farm level, contaminated soil and wastewater were found to be the main risk factors for produce quality. The higher impact of soil contamination on farm produce quality in the rainy season than in the dry season could be due to the frequent splashes of soil on produce arising from rainfall, and has been suggested by others [141, 166]. The use of poultry manure did not show significant association with produce quality, though this could be due to the fact that data was only collected on reported manure use, within the last four weeks. Similar findings were reported by Amoah *et al*. [67, 141] during field trials in Accra, where direct wastewater use was the major risk factor for produce contamination, with contaminated soil and poultry manure identified as other potential sources of contamination. In this field trial, the use of manure increased the levels of faecal coliform on lettuce cultivated on average by tenfold, while the use of wastewater increased faecal coliform levels 0 -100 fold. The high use of manure in this study was similar to those reported in previous studies (73% to 98%) in Accra and Kumasi, where manure was found to be highly contaminated with faecal coliforms ranging from 1.0 10^3 to 1.0 10^8/100 g [48, 67, 167]. Poultry manure is recommended only as soil amendment if it has been adequately dried, i.e. composted aerobically to levels between 60°C and 80°C, and for at least 15 days before application [157], something which was not reported to be practiced by farmers. Restrictions on the use of untreated wastewater, the adoption of crop restriction, together with drying of poultry manure remain the best ways to ensure food safety in Accra. However, these measures are difficult to implement in resource constrained countries as a result of high cost, lack of alternative sources of irrigation water, and farmers' unwillingness to cultivate non-vegetables, or non-food crops for loss of profits. In countries where good quality irrigation water sources are unavailable in the short to medium term, interventions that require less restrictions and minimal financial investment from farmers are recommended as farmers find these more attractive to adopt [71]. These interventions include agricultural practices that limit produce contact to sediments such as controlled fetching of irrigation water from sedimentation ponds, the use of watering cans fitted with caps or fabric filters to reduce splashes of contaminated soil on produce, and the use of simple filters [71, 129, 166]. These interventions, however, need to be tested widely to assess their implementation successes and challenges. Education on the proper use of manure as well as local authorities' collaboration and support to farmers to gain access to alternative fertilizers, and water sources such as wells are also significant measures that can be taken to mitigate exposure and health risks.

3.5 Conclusion

The results of this study suggest that the use of untreated wastewater plays a significant role in influencing health risks arising from produce contamination at the farm level but its role in influencing consumer risks at markets and kitchens remains unclear. Though produce was contaminated at all entry points of the food chain, the street food domain was found to be the most critical domain for risk reduction interventions due to the high levels of contamination found on salad food, the large number of consumers who patronise street food, and the fact that it presented the highest risk to consumers based on the indicator QMRA models. The study recommends field trials, or related studies to establish the effect of wastewater irrigation on produce quality and hence consumer risks at markets and kitchens and whether this effect is significant in terms of public health. It also recommends an assessment on the influence of produce washing practices at markets, and vendors' preparation, handling and management of salad during sales, on the quality of salad produce. Lastly, further studies are needed to determine the presence and concentrations of pathogens to help improve the quality of QMRA estimates. The study concludes that in as much as interventions at the source of production (farms) may result in significant positive public health impact, adequate hygienic practices at markets but especially at points of consumption (food kitchens) are regarded as more effective in terms of cost, ease of implementation and above all public health significance.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Prince Antwi-Agyei
Principal Supervisor	Dr Jeroen Ensink
Thesis Title	Wastewater use in urban agriculture: an exposure and risk assessment in Accra

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	My supervisor and I designed the study. I developed all data collection tools and collected field data. I also performed all statistical analysis and was the lead author for the manuscript.

Student Signature: ____________________ **Date:** ______________________

Supervisor Signature: __________________ **Date:** _____________________

Chapter 4: Risk perceptions of wastewater use for urban agriculture in Accra, Ghana

Photo 4.1: A stream receiving municipal wastewater and used by urban agriculture farmers in Accra, Ghana

Abstract

Poor food hygiene is a significant risk to public health globally, but especially in low and middle-income countries where access to sanitation, and general hygiene remain poor. Food hygiene becomes even more pertinent when untreated, or poorly treated wastewater is used in agriculture. Where wastewater is used in agriculture the WHO recommends the adoption of a multiple-barrier approach that prescribes health protective measures at different entry points along the food chain. This study sought to assess the knowledge and awareness of wastewater use for crop production, its related health risks, and adoption of health protective measures by farmers, market salespersons and consumers using questionnaires and focus group discussions.

In the period from September 2012 to August 2013, 490 participants were interviewed during two cropping seasons. The study found that awareness of the source of irrigation water was low among consumers and street food vendors, though comparatively higher among market vendors. In contrast, health risk awareness was generally high among salespersons and consumers, but low among farmers. The study found that consumers did not prioritize health indicators when buying produce from vendors but were motivated to buy produce, or prepared food based on taste, friendship, cost, convenience and freshness of produce. Similarly, farmers' awareness of health risk did not influence their adoption of safer farm practices. The study recommends the promotion of interventions that would result in more direct benefits to both producers and vendors, together with hygiene education and enforcement of food safety byelaws in order to influence behaviour change, and increase the uptake of the multiple-barrier approach.

Keywords: Ghana, wastewater irrigation, risk perceptions, behaviour change, produce

4.1 Introduction

Globally, diarrhoea remains one of the leading causes of death in both adults and children [168]. The risk factors for diarrhoeal diseases are multi-faceted, but an estimated 94% of cases are attributed to environmental factors which include unsafe drinking water, poor sanitation and hygiene [169]. For example, an estimated 502,000 and 280,000 deaths in 2012 were associated with inadequate water and sanitation respectively from a total of 1.5 million diarrhoea related deaths [170].

The role of improved food hygiene in the transmission of diarrhoeal disease is under researched. Food hygiene interventions, especially for food that can be eaten uncooked (e.g. salads) remain a challenge, especially in low and middle-income countries (LMICs) where access to sanitation, and general hygiene are poor. Food hygiene interventions are further complicated in countries where water is scarce, and wastewater is used for agricultural production. The use of wastewater in agriculture has been associated with diarrhoeal disease, and helminth infections in both farmers and consumers [31]. However, post-harvest contamination could pose even bigger health risks than when untreated wastewater is used in agriculture [2, 65].

To safeguard human health when wastewater is used in agriculture, the World Health Organisation (WHO) has developed guidelines to regulate the use of wastewater. The 2006 revision of the WHO guidelines recommends a multiple-barrier approach to protect consumer and farmer health [3]. The multiple-barrier approach stipulates health protection measures at different entry points along the food chain, and is particularly recommended for countries where wastewater is used without treatment [3]. Currently, the implementation and uptake of the proposed non-wastewater treatment protective measures has been slow due to attitudes and perceptions of farmers, retailers and consumers [171]. In addition, there is inadequate field-based evidence on the effectiveness and efficacy of these risk reduction measures especially regarding on-farm measures and hygienic food marketing and food preparation at markets, homes and kitchens in low and middle-income countries [10]. Studies have suggested that interventions are more likely to be successful when they are designed to incorporate the target groups' perceptions, attitudes, suggestions/knowledge and constraints [71, 172]. Education and awareness creation on the health risks of wastewater irrigation has been recommended as one of the health protective measures [10, 70] but prior to undertaking this, it is important to understand the diverse food safety issues and perceptions relevant to producers and consumers [171]. Health risk perceptions, describe the subjective judgement of people to health risk, or behaviours and could be triggered by factors such as tradition, family pressure, community norms, time pressure and inconvenience [72, 171]. This study sought to assess how farmers, crop handlers and consumers' knowledge and awareness of health risks of produce irrigated by wastewater influence their buying, consumption and food hygiene practices.

4.2 Method

The study adopted a mixed method approach including the use of semi-structured interviews, and focus group discussions. A total of 490 participants, including wastewater farmers, market salespersons, street food vendors and public and domestic consumers of produce were interviewed from October 2012 to December 2012 (dry season), and from June to August 2013 (wet season).

4.2.1 Study sites

There are an estimated 160 hectares, over seven major sites of farmland, irrigated by wastewater in Accra, Ghana. Each of the major sites has between 60 and 200 agricultural workers. The predominant sources of irrigation water at these sites are municipal wastewater sources including: open drain water, and dug-outs (ponds). Farmers apply water to their crops, which include: lettuce, cabbage, spring onions, and a host of other local vegetables using watering cans. Produce from these farms is sold to market vendors, street food vendors, and restaurants within Accra. For markets, the study focused on central markets, which have the largest population of vendors and customers, and serve as wholesale distribution centres for traders dealing in salad vegetables [133]. Unlike restaurants and hotels, the street food sector in Accra is largely informal, and hence difficult to regulate. An increasingly popular category of street food vendors in Accra is the "check-check" (fast food) seller. These vendors are found in open spaces with decorated and stylishly mounted kiosks that sell fast food especially in the evenings. Most of these food vendors have no permit, or hygiene certificates from the public health departments [173].

4.2.2 Sample size and selection

For a faecal exposure assessment study, 80 farmers were randomly selected for observation, and later interviewed (Chapter 2). Similarly, 80 market salespersons from three central markets that sold salad crops, in particularly lettuce and cabbage were randomly selected; using their market stalls, or sheds numbers for selection (Chapter 3). Street food consumers (160) and domestic consumers (160) of salad vegetables were also included in the study, and were selected when they bought their food, or raw produce within the observation period at the selected sites (Chapter 3). Food vendors in the selected communities in Accra could not be easily identified as they were not registered. Consequently, the 160 street food consumers were divided over 30 street food vendors ("check-check" sellers) who were randomly recruited from an already generated numbered list of food vendor stalls previously identified by a transect walk with community leaders in two communities. One community was a planned settlement (Alajo), while the other was a squatter settlement (Old Fadama). Restaurants and hotels in Accra where salad was served to the public were also included in the study during the rainy season, and were selected on the basis of their "star" rating, location and popularity. The selection was done in collaboration with the Ghana Food and Drugs Authority (FDA) from their database of restaurants and hotels.

4.2.3 Data collection

Questionnaire

Questionnaires were verbally administered to street food vendors, and consumers of fast food between 18:00 and 21:00, while domestic consumers, farmers and market vendors were interviewed from 7:00 to 10:00. Questionnaires were administered to participants immediately after any observation was carried out. With farmers, questions dealt with defaecation practices, and food hygiene practices at farms (Annex 2a), while market and street food vendors were interviewed about awareness of the source of irrigation water, health risks associated with wastewater irrigation, and whether this awareness, or the source of irrigation water influenced their buying and consumption of salad vegetables (Annex 2b & 2d). Interviews with chefs took place at hotels and restaurants, and covered among other topics, the sources of produce for salads, and methods of treating salad (Annex 1e). Only the chef who prepared the salad at the time of sample collection was interviewed. Questionnaires for street food, and domestic consumers included salad consumption frequency, factors that influenced their purchase and health risk awareness associated with wastewater use for irrigation (Annexes 2c & 2e).

Focus group discussion

Three focus group discussions (6 per group) were held each with market vendors and farmers who were not included in previous interviews, or observations. Focus group discussions explored similar topics as the questionnaires, and were meant to provide complementary information (Annexes 3a & 3b). Where appropriate, participants for focus group discussions were selected to ensure a similar sample to those questioned, or observed on the basis of gender, years of working experience, or religion. There were three researchers for each focus group discussion, the lead conductor and two note takers. All focus group discussions were audio recorded and later transcribed.

4.2.4 Data Analysis

All data were analysed using STATA 12 (StataCorp LP, College Station, USA). The association between participants' knowledge and awareness of wastewater use, health risk, and consequently buying and consumption of salad, together with the adoption of health protective measures was assessed using Pearson Chi-square test, and logistic regression models. Awareness of health risk and buying, or consumption of salad were the main outcomes while participants' personal characteristics, awareness of source of irrigation water/health risk or source of produce were the main exposures. Awareness of health risk meant participants had heard that wastewater use could result in disease outcome, while awareness of source of irrigation water meant that participants had been told, or knew about the various types of water sources (taps/piped water, drain water, ponds) used to irrigate crops. Awareness of health risk was confirmed as knowledge of the risk if participants were able to mention correctly a disease associated with the consumption of wastewater irrigated produce. Odds ratios (OR) were used to measure the association between exposures and binary outcomes in both univariable and multivariable logistic regression models. Only factors that were significant at 10% in the univariable analysis were included in the multivariable logistic model. Statistically significant associations between exposures and outcomes in the multivariable analysis were measured at 5% significance level using the likelihood ratio test. Data from focus group discussions were transcribed verbatim using Microsoft office word, and thematic content coding was used to analyse the data from predetermined themes [174]. Interaction of individual responses among the groups was taken into account and group was used as the unit of analysis.

4.2.5 Ethical Approval

Ethical approval was received from the Ethical Review committees of the London School of Hygiene and Tropical Medicine (LSHTM, reference number - 6236) in the UK and from the Noguchi Memorial Institute of Medical Research in Ghana (Reference number – DF22). The study was also explained and agreed to by local leaders and written informed consent (verbal in some cases) obtained from each individual participant for questionnaires and focus group discussion.

4.3 Results

4.3.1 Characteristics of study participants

The majority (95%) of farmers were males with an average age of 40 years. Almost a third (31%) of farmers had no formal education, and urban agriculture provided the main source of income for nearly 80% of farmers. Market salespersons, in contrast, were predominantly female (95%) with nearly 90% above 30 years of age. Street food vendors were mostly males (83%), had some formal education (97%), and the large majority (76%) was younger than 30 years of age. The consumers of check-check food, were 61% males with the majority (87%) below 30 years (range 11 – 54 years). Buyers of produce at markets were mostly female (84%) with an average age of 37 years.

4.3.2 Awareness of sources of irrigation water, and produce

Among all participants, awareness of the source of irrigation water used by farmers for vegetable cultivation was highest among market vendors (66%, Figure 4.1). Market vendors also stated that produce was more likely to be irrigated with wastewater if it was cultivated within Accra than outside Accra – *"Yes, we are aware of the irrigation water farmers use and some customers will even ask you where you get your lettuce from. Some will not buy if you tell them the lettuces are from Accra"* (FGD). Contrary to market vendors, a far lower proportion of street food vendors (21%) and consumers of street food (30%) claimed being aware of the sources of irrigation water.

The questionnaire also showed that farmers in Accra were the biggest suppliers of lettuce at the markets (40%), and that more than a third (38%) of vendors bought their vegetables directly from farm gates in Accra, while the rest bought from wholesale, and retail markets within, and outside Accra. Market vendors in focus group discussions also claimed good awareness of the origin of produce: *"We know of the source of water farmers used to irrigate vegetables in Accra – you can even smell it when you buy the produce*". At markets, less than half of domestic consumers (44%) reported being aware of the source of produce they bought at markets (Table 4.1). All street food vendors reported buying their produce from wholesale, or retail markets, though observations showed that some vendors bought directly from wastewater irrigated farms. Chefs at restaurants, and hotels mostly bought salad produce directly from farms, or third party suppliers (55%); with the rest buying from wholesale/retail markets (35%), or supermarkets (10%).

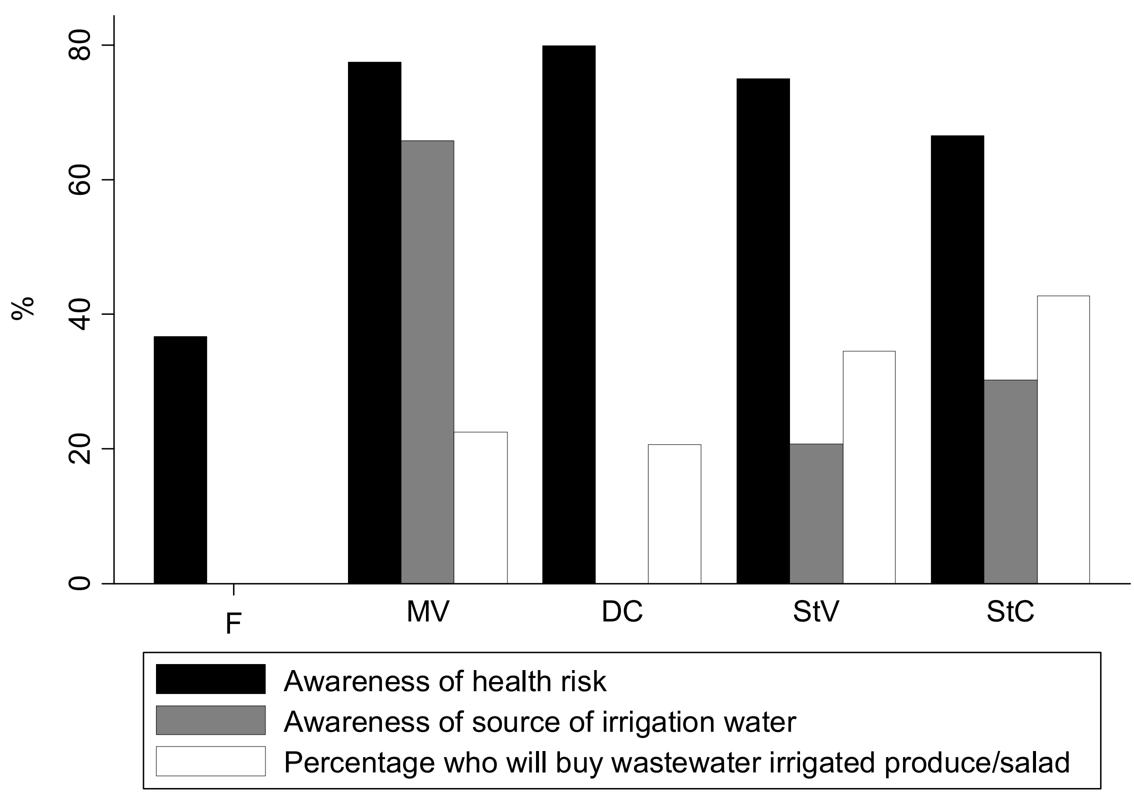

Figure 4.1: Wastewater irrigation and health risk awareness and perceptions
MV=market vendors, DC= domestic consumers (produce buyers at markets), StV=street food vendors, StC=street food consumers, F=farmers

Table 4.1: Determinants of awareness of wastewater irrigation health risk among domestic consumers of salad

			Univariable analysis		Multivariable analysis	
Exposure	N=159	Awareness of risk (%)	OR^c (95% CI)	P* - value	OR^a (95% CI)	P† - value
Awareness of source of produce						
No	89	70	1.0	< 0.001	1.0	0.002
Yes	69	93	5.6 (2.02, 15.40)		5.1 (1.82, 14.16)	
Religion						
Muslim	27	63	1.0	0.02	1.0	0.08
Christian	132	83	2.9 (1.19, 7.27)		2.3 (0.91, 6.01)	
Gender						
Female	135	80	1.0	0.93	NA	NA
Male	24	79	0.9 (0.33, 2.77)			
Age group						
≤ 30	57	79	1.0	0.50	NA	NA
31 - 40	49	84	1.4 (0.52, 3.76)			
41 - 50	37	73	0.7 (0.28, 1.94)			
> 50	17	88	2.1 (0.41, 10.21)			
Occupation						
Gov. worker	17	76	1.0	0.70	NA	NA
Traders	81	79	1.2 (0.33, 4.01)			
Vocational	49	80	1.2 (0.32, 4.49)			
Others	12	92	3.4 (0.33, 34.92)			

OR^c = crude odds ratio, OR^a = adjusted odds ratio, NA = not applicable
**P-value from logistic regression.*
† p –value calculated from likelihood ratio test

4.3.3 Knowledge of health risks associated with wastewater irrigation

Among all study participants, awareness of health risk associated with wastewater irrigation was highest among domestic consumers (80%) of salad vegetables, while farmers were the least (37%) likely to associate wastewater use to health risks - *"I don't think it is possible to get any disease after consuming produce irrigated with drain water. We even eat some of the raw lettuce on the farm, and we don't always wash them, let al.one use disinfectant" (FGD).* Farmers claimed to wear boots to avoid cuts rather than to prevent contact to contaminated soil, or wastewater. "*We are used to walking barefoot and we don't think there are any health effects with that. Farmers wear boots to protect themselves from cuts from broken bottles or other sharp materials. Farmers who are at sections of the farm where these sharp materials are normally wear the boots"* (FGD). Farmers' age, sex and religion had no significant association with wastewater risk awareness. The only factor associated with farmers' awareness that wastewater irrigation carried health risks was higher education levels, with farmers with primary education, or secondary education almost 5 and 8 times as likely to associate health risks to wastewater use, as farmers without formal education ($p = 0.05$). Rather than health risks, farmers were more concerned about getting support from government in terms of provision of seeds, subsidies for fertilisers and other agro-chemicals and provision of land for farming or assurance of land security. *"Government should take charge and subsidize the cost of fertilizers and vegetable seeds, since these are sold at a far higher price on the private market. It even becomes difficult to get supply at certain times" (FGD).* Farmers in a focus group discussion also attributed the above problems to the weak relationship that exists between local authorities and farmer associations.

Among market vendors, being aware of the source of irrigation water was associated with higher awareness of health risk (OR = 4.6, $p = 0.06$), though this association was non-significant (OR = 4.7, $p = 0.12$) when controlled for gender, age, education and religion. For domestic consumers, awareness of the source of produce was significantly associated with a higher awareness of wastewater related health risk after controlling for religion (OR = 5.1, 95% CI = 1.8 – 14, $p = 0.002$, Table 4.1). Gender, age and occupation of domestic consumers had no significant association on their awareness of health risk while for religion, Christians had a slightly (borderline significance) increased awareness compared to Muslims (OR = 2.3, 95% CI: 0.91 – 6.01, $p = 0.08$). The majority (75%) of street food vendors also associated the consumption of wastewater irrigated produce with health risks. No association was found between risk awareness and vendor characteristics. Similar to vendors, awareness of wastewater health risks was also high among street food consumers. After controlling for religion and awareness of the source of irrigation water, male consumers of street food were almost three times ($p = 0.02$) as likely to be aware of wastewater health risk as female consumers (Table 4.2). Similarly, consumers' awareness of the source of irrigation water used for vegetable cultivation was associated with a higher awareness of health risk. Age and occupation of street food consumers had no significant influence on their awareness of wastewater health risk.

In terms of knowledge of diseases, just over 50% of all participants did not associate any health risks with exposure to wastewater, or failed to correctly mention a disease associated with exposure to wastewater (Table 4.3). Knowledge of wastewater related diseases was highest among domestic consumers with 66% correctly identifying diseases such as diarrhoea/cholera and worm infections, but lowest among farmers (26%). Although awareness of wastewater health risks was high among street food consumers, it did not necessarily translate into knowledge of wastewater use related diseases, with 35% of those who were aware of the health risks unable to correctly mention a disease associated with exposure to wastewater.

Table 4.2: Determinants of awareness of wastewater irrigation health risk among street food consumers of salad

			Univariable analysis		Multivariable analysis	
Exposure	N=158	Awareness of risk (%)	OR[c] (95% CI)	P* – value	OR[a] (95% CI)	P† - value
Religion						
Muslim	101	55	1.0	< 0.001	1.0	0.001
Christian	56	88	5.9 (2.42, 14.16)		4.8 (1.96, 12.02)	
Gender						
Female	63	54	1.0	0.007	1.0	0.02
Male	95	75	2.5 (1.28, 4.97)		2.8 (1.19, 5.15)	
Age group						
≤ 20	46	65	1.0	0.84	NA	NA
21 - 30	91	68	1.1 (0.54, 2.41)		NA	
> 30	21	62	0.9 (0.30, 2.53)		NA	
Occupation						
Traders	56	38	1.0	0.38	NA	NA
Student	37	22	2.2 (0.84, 5.63)		NA	
Vocational	15	27	1.7 (0.47, 5.85)		NA	
Scrap dealer	22	41	0.9 (0.32, 2.37)		NA	
Other	28	39	0.9 (0.37, 2.35)		NA	
Awareness of source of irrigation water						
No	110	60	1.0	0.09	1.0	0.05
Yes	47	81	2.8 (1.24, 6.40)		2.4 (1.0, 5.77)	

OR[c] = crude odds ratio, OR[a] = adjusted odds ratio, NA = not applicable
**P-value from logistic regression.*
† p –value calculated from likelihood ratio test

Table 4.3: Proportion of participants who mentioned diseases associated with exposure to wastewater irrigation

Type of disease	Farmers (N = 80)	Market vendors (N = 40)	St food vendors (N = 29)	Produce buyers (N = 159)	St. food consumers (N = 158)	All participants (N = 466)
No risks	64%	23%	31%	20%	33%	33%
Those aware of health risks						
Diarrhoea	19%	35%	24%	41%	21%	29%
Cholera	5%	18%	24%	23%	17%	17%
Worm infection	2.5%	2%	7%	2%	5%	3.4%
Non-related ones	3.7%	15%	14%	8.8%	8%	8.4%
Cannot tell	6%	7%	0%	5.0%	16%	9%

4.3.4 Factors influencing consumers to buy produce or prepared salad food.

Despite the high awareness of health risk, street food consumers did not prioritise health indicators when buying food. Only 2% of consumers chose a vendor based on food safety reasons (Figure 4.2). Instead, consumers were more concerned about the taste of the food (46%), and the proximity of their house/work place to vending sites (19%). A higher number of consumers also seemed satisfied with sanitation conditions (82%), and how vendors prepared their salad (71%), despite the poor environmental and food hygiene practices at the vending sites. Similar to street food consumers, there was high variability in domestic consumers' attitudes about indicators used to assess produce safety. Buyers' choice of a vendor for produce was based primarily on friendship/good customer care (28%) and good price (20%) (Figure 4.3). Health indicators like clean environment, and how well produce had been displayed were lower priority for consumers (Figure 4.3). Domestic consumers of produce also seemed satisfied with how produce was displayed (82%), and the general sanitation at vending sites (62%). Market vendors in a focus group discussion stated that they were most concerned about keeping their vegetables fresh, and ensuring environmental cleanliness in order to attract customers.

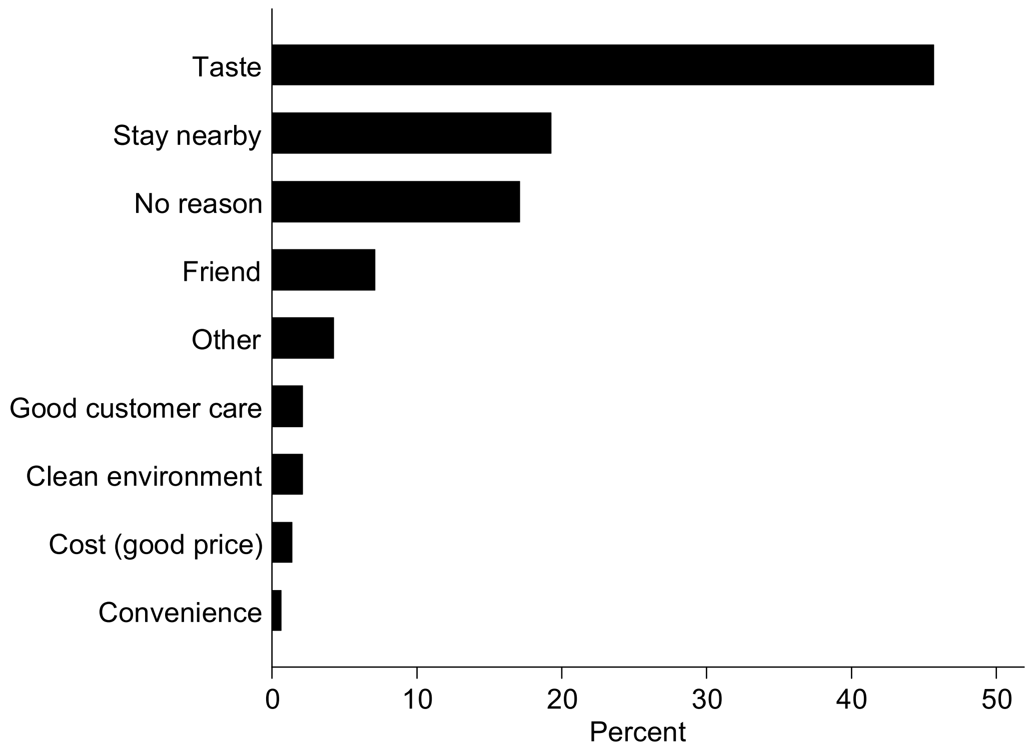

Figure 4.2: Main factors influencing street food consumers to buy prepared salad from vendors

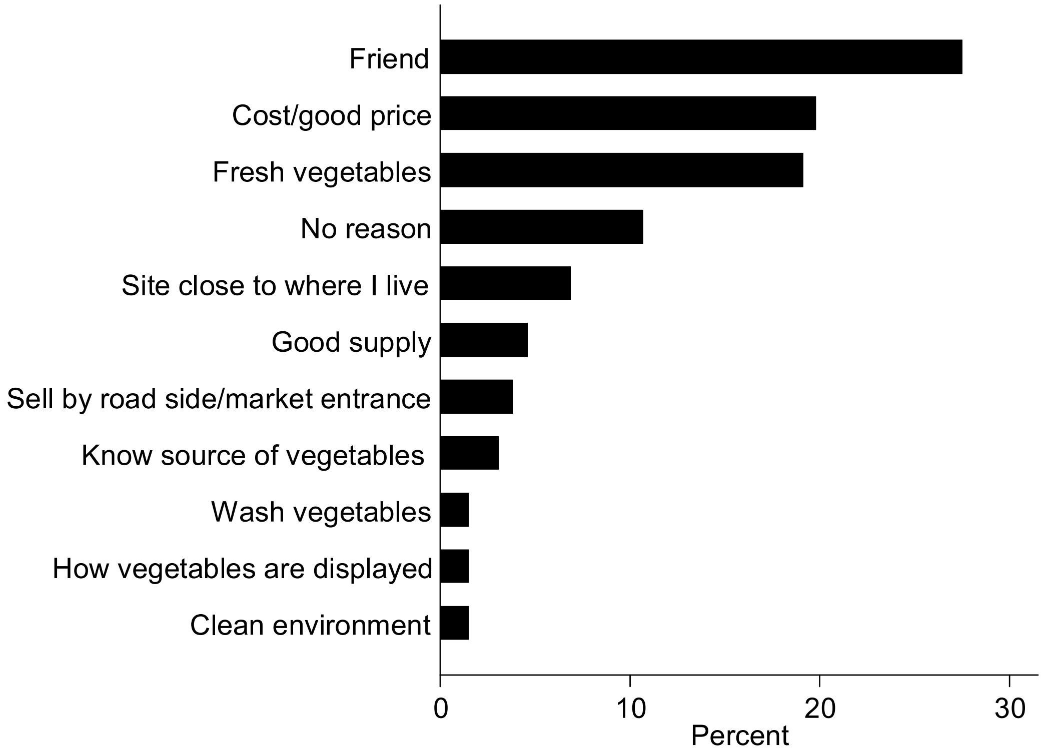

Figure 4.3: Main factors influencing domestic consumers to buy produce from market vendors

4.3.5 Salad consumption patterns

The large majority of street food consumers (90%) reported to consume salad for an average of 4 times a week, while those (51%) who consumed salad at home did so for 2.8 times per week. Consumers of street food ("check-check") attributed the taste/likeness of the food (49%) and convenience (30%) as the main motivator for their consumption; aside from cost (11%). Gender, occupation, age group and religion of consumers had no significant influence on their consumption of salad. After controlling for occupation, street food consumers, who were aware of wastewater related health risk were 2.2 times less likely to buy salads, or add salad to their food, if they knew the produce was wastewater irrigated (95% CI = 1.1 – 4.7, p = 0.03, Table 4.4). Despite this observation, over 40% of street food consumers indicated that they would still consume salads even if the produce were wastewater irrigated (Figure 1). Street food consumers would often quote the Ghanaian proverb *"Ani ahu a, ɛnyɛtan"* to describe their reaction to the fact that farmers may be using wastewater for irrigation or vendors not preparing their salad in a hygienic way but *"If the eye does not see these things, it does not make the food disgusting"*. For domestic consumers, buying produce was not influenced by awareness of health risk, nor was it influenced by the source of produce (Table 5). Buying of produce was, however, strongly associated with knowledge of the source of irrigation water used for vegetable cultivation with those who were aware of the source of water 8 times less likely to buy wastewater irrigated produce (Table 4.5).

Table 4.4: Determinants of buying wastewater irrigated produce used for salad among street food consumers

Exposure			Univariable Analysis		Multivariable analysis	
	N=160	**Buy (%)**	**OR^c (95% CI)**	**P*-value**	**OR^a (95% CI)**	**P†-value**
Religion						
Muslim	100	45	1.0	0.38	NA	NA
Christian	58	38	0.7 (0.39, 1.45		NA	
Gender						
Male	96	35	1.0	0.03	1.0	0.71
Female	63	52	2.0 (1.05, 3.83)		1.2 (0.53, 2.53)	
Occupation						
Trading	57	47	1.0	0.01	1.0	0.01
Vocational	15	60.	1.7 (0.52, 5.30)		2.8 (0.55, 5.82)	
Student	36	53	1.2 (0.54, 2.86)		1.4 (0.59, 3.32)	
Scrap dealer	22	18	0.2 (0.07, 0.82)		0.2 (0.06, 0.75)	
Other	29	28	0.4 (0.16, 1.11)		0.4 (0.15, 1.11)	
Age group						
≤ 20	45	53	1.0	0.07	1.0	0.22
21 - 30	93	41	0.6 (0.30, 1.24)		0.76 (0.32, 1.81)	
> 30	21	24	0.3 (0.09, 0.87)		0.31 (0.08, 1.24)	
Awareness of wastewater health risk						
Yes	105	38	1.0	0.10	1.0	0.03
No	52	52	1.8 (0.90, 3.44)		2.2 (1.09, 4.66)	

OR^c = crude odds ratio, OR^a = adjusted odds ratio, NA = not applicable
**P-value from logistic regression.*
† p –value calculated from likelihood ratio test

Table 4.5: Determinants of buying wastewater irrigated produce at markets among domestic consumers of vegetables†

Exposure	Obs N = 160 (%)	Buy (%)	OR (95% CI)	P** - value
Religion				
Christian	133 (83)	19*	1.0	0.22
Muslim	27 (17)	30	1.8 (0.72, 4.63)	
Gender				
Male	25 (16)	16	1.0	0.52
Female	135 (84)	22	1.4 (0.46, 4.52)	
Occupation				
Trading	82 (51)	21	1.0	0.32
Government worker	17 (10.6)	0.0	-	
Vocational	49 (31)	23	1.1 (0.47, 2.61)	
Others	12 (7.5)	42	2.7 (0.77, 9.68)	
Age group				
≤ 30	57 (35.6)	25	1.0	0.55
31 - 40	49 (31)	23	1.1 (0.43, 2.74)	
40 - 50	37 (23)	14	0.6 (0.19, 1.83)	
> 50	17 (10.6)	29	1.6 (0.46, 5.30)	
Awareness of health risk				
No	32 (20)	19	1.0	0.83
Yes	127 (80)	21	1.1 (0.42, 2.99)	
Awareness of source of produce				
No	90 (57)	19	1.0	0.51
Yes	69 (43)	23	1.3 (0.60, 2.80)	
Influenced by source of irrigation water				
Yes	116 (73)	10	1.0	< 0.001
No	43 (27)	49	8.3 (3.55, 19.26)	

** Percentage of Christians who would buy wastewater irrigated produce*
*** p-value calculated from logistic regression*
†No Multivariable model as only one parameter (source of irrigation water) was significantly associated with buying wastewater irrigated produce in the Univariable logistic model.

4.4 Discussion

The study found that awareness of the source of irrigation water used for crop production was low among consumers and street food vendors, though higher among market vendors. Similarly, awareness of wastewater irrigation health risk was relatively low among farmers, but high among vendors and consumers, though this did not necessarily influence their decision to buy produce, or consume salad. The study further showed that awareness of health risk did not necessarily mean knowledge of the actual health risk, or source of risk, nor in the use of health protective practices among farmers, vendors and consumers.

4.4.1 Awareness of the source of produce

In order to protect human health from the possible negative health impact of wastewater use in agriculture, the WHO has promoted a multi-barrier approach. The success of this approach will depend largely on whether consumers know exactly where their produce originated from, and what it was irrigated with, but also whether they know what they can do to minimise health risks. In this study, awareness about where and what produce was irrigated with was low among consumers but higher among vendors of produce at local markets. The low awareness of the sources of irrigation water, or the source of produce was also corroborated by a review study that found that most buyers and consumers in Accra were actually unaware of the sources of produce, or the use of polluted irrigation water since they rarely ask these from vendors [2]. It is estimated that 50% to 90% of vegetables consumed by urban dwellers in West Africa are wastewater irrigated [99], which, inadvertently suggests that it might be inadequate to rely on the town, or country to determine the source of irrigation water, as was done by market vendors in the current study. This study found that vendors and consumers rarely considered the source of produce when buying produce but rather were influenced by other indicators particularly the freshness of the vegetables, confirming other findings in Ghana and in the United States [141, 175]. Urban agriculture is often promoted as a way to ensure a livelihood for the urban poor, and to grow fresh produce close to the places where it is sold to consumers [10, 49, 176]. The short distances to mark*et al*.so mean that transport and refrigeration cost are low, making the produce cheaper. This is a win-win situation for farmers, market vendors and consumers. Telling consumers where the produce originated from might turn some consumers, as was shown by this study, away from buying this produce, as the use of domestic or municipal wastewater holds not only health risks, but is also a taboo in Ghana [71]. This will result in a loss of revenue, and making it unlikely that market and street food vendors will advertise the origin of their produce. However, for the multi-barrier approach to function successfully, this is what is required. This is not a task for farmers or market vendors, but involvement of local health, agricultural agencies and other key institutions is required.

4.4.2 Health risks awareness among farmers

Farmers in this study reported the lowest health risk awareness when it came to the use of wastewater in irrigation, even though they should be the first 'implementers' of food safety measures in the multiple barrier approach. Keraita *et al*. [71] and Qadir *et al*. [2] have reported similar findings from studies in Ghana attributing it to illiteracy, lack of adequate information and resources, and the fact that farmers have been exposed to poor sanitary conditions for most of their lives, and therefore tend to accept the risks for the benefits of their occupation. It is also possible that farmers in low and middle income countries may have developed some level of immunity or resilience to pathogens from wastewater, and hence their reported low awareness of health risk. This study, however, showed that improved education was strongly associated with higher awareness of wastewater related health risk. Awareness of health risk associated to wastewater use was higher among wastewater farmers in Kenya (53%) and Bulawayo, Zimbabwe (70%), [70, 177]. In Pakistan, farmers irrigating with untreated wastewater stated that there were no negative health impacts associated with wastewater use [30], and there, as well as in Ghana this was contributed to a defensive mechanism to protect their livelihood, and not necessarily a lack of knowledge of health risk, or protective measures [71]. Farmers are often acutely aware that the use of wastewater for vegetable cultivation is illegal and frowned upon by society and therefore in order to keep their job; tend to under report the associated health risk, or argue that their source of irrigation water poses no risk [71]. However, farmers are willing to adopt risk reduction measures to avoid further pressure from city authorities and the media, as a study in Accra found [71], though land insecurity has been suggested as one of the key reasons for farmers not investing in measures that could reduce health risks, like drip irrigation, use of boreholes and on-farm sedimentation ponds [10, 178].

4.4.3 Adoption of protective measures

Several reasons were found for the low adoption of health protective measures when buying produce, or prepared food, which included low awareness of the sources of irrigation water and a much lower priority of health parameters, including the hygiene conditions at food vending sites. The study found that although awareness of health risk related to wastewater use in agriculture was high, it did not necessarily translate into the adoption, or usage of health protective measures.

The fact that risk awareness, and knowledge of risks, do not translate into healthy behaviour has been shown in past hygiene programmes; with several studies having shown that the presence of soap in household and knowledge of when to wash hands, did not translate in high hand washing rates at key times [179, 180]. A study in Ghana found high education levels among mothers (73%), and 55% knew of at least two of the three key times when to wash hands with soap, though the prevalence of hand washing with soap was very low

(3.6%) [179]. Biran *et al*. [181] have also speculated that efforts to change hand washing behaviours at large scale have achieved little success because they relied on communicating health benefits of hand washing with soap, rather than giving more attention to the effect of emotional drivers. Our results also show that being aware of the health risk did not necessarily influence the buying of produce or consumption of salad particularly among domestic consumers. The first possible explanation was that most salespersons and consumers were unaware of the sources of produce, or the quality of irrigation water, confirmed by the fact that those that were aware, were less likely to buy wastewater irrigated produce. However most vendors and consumers were motivated to buy produce, or consume salads using indicators unrelated to food hygiene and safety, like friendliness of the vendor, and taste. A further reason stated was a lack of time or money. These findings are similar to those from central Ghana (Kumasi) where consumers relied mainly on neatness, appearance and trustworthiness of the vendor, in addition to cost and accessibility in their choice for a food vendor [182]. Interestingly, both neatness and trust were construed by vendors and consumers using indicators of different dimensions to the normal classical definition of these words [182]. Convenience, or a lack of time is an important driving force to buy produce at one particular place, or to use food vendors where the food quality is unknown.

The findings of this study suggest that relying on health indicators to raise awareness or promote the uptake of health protective measures may not be sufficient to influence positive behaviour change. Campaigners of hand washing promotion programmes and the community led total sanitation (CLTS) approaches have suggested that the use of emotional drivers like disgust, habit formation, nurture and affiliation could better engender long lasting behaviour change than the promotion of health benefits which could only be effective in cases of disease epidemics [181, 183, 184].

Aside from emotional drivers, the promotion of interventions that would create more direct benefits to producers, vendors and consumers must be prioritised. These interventions are likely to be successful if they are implemented using participatory approaches that build upon existing practices of farmers and vendors and should require minimal changes to current practices and low capital investment from farmers and salespersons [71]. Farmers in this study felt a lack of support from government in respect to agricultural extension support. Agricultural extension programmes could also incorporate food hygiene, and advice on how to minimize negative health impact to consumers and farmers, and possibly provide support to install clean water points, where produce can be washed. The current relationship between farmers' associations, and local authorities is weak as a result of insecure tenure arrangements, and a lack of representation and communication. In Kenya, a study found that farmers who received support from NGOs, and had access to credit were more likely to adopt innovative risk reduction interventions to minimize health risks linked to wastewater irrigation [70].

Similar to interventions to increase the uptake of risk reduction measures at the farm level, collaborative approaches are needed to increase trust between vendors and local authorities, the media and consumers regarding food safety. One way of handling this is to develop effective communication channels between farmers, vendors and consumers on risk reduction measures based on the multi-barrier approach. The use of the Ghana Water and Sanitation journalist association could be a suitable way to bridge this gap. Local authorities should also require from vendors to join vendor associations in order to benefit from hygiene and behaviour change education programs. Leading members in such associations can be used as role models, and agents of change in the vendor business. Currently, most market and street food vendors have no experience in hygiene training but the award of training certificates on food hygiene and safety could increase consumer confidence, and trust in vendors, which possibly could benefit vendors with increased sales. The use of regulations is widely recognised as important for public health safety but they should be practically feasible within the existing local constraints and challenges faced by producers and vendors [185]. Consumers have a significant influence on vendors and farmers, and can contribute to the adoption of safer practices by buying from only those who adhere to hygienic requirements. Market vendors can also capitalise on societal pressure on farmers for safer food by branding safer production sites with names associated with accepted norms such as 'neat' and 'clean' similar to market vendors' supposed preference of carrots from Togo than those from Ghana [153].

4.5 Conclusion

The multiple-barrier approach is a key component of the WHO guidelines but can only function effectively if relevant stakeholders, particularly national institutions, are committed to play their clearly defined roles and responsibilities of ensuring public health safety. For farmers and salespersons, and also for consumers, the adoption of the approach may be influenced in part by the recognition of their vulnerability to health risk from wastewater, or wastewater irrigated produce, and what risk avoidance methods they need to practice to protect themselves; but more importantly the direct benefits they will get by adhering to the approach. The results from this study showed that even when people were aware of the health risk associated with wastewater irrigation, or were aware of the source of produce, it was not sufficient to influence their adoption of health protective measures, or influence them to buy and consume wastewater irrigated produce. On the contrary, people were likely to change behaviours, including the use of risk reduction measures if they knew the actual source, or type of irrigation water used for vegetable production. The study findings also seem to suggest that salespersons and consumers of salad vegetables were less concerned about health risks associated with wastewater irrigated produce, and even in cases where they expressed concern, they rarely relied on health indicators in their decision to buy produce or consume salad. Similarly, farmers' awareness of health risk alone was not a significant factor influencing their adoption of risk reduction measures. From the foregoing, it is clear that in order to reduce health risks, and to increase the uptake of the multiple-barrier approach, interventions that could more directly impact benefits (especially economic benefits) to producers, salespersons and consumers of salad crops should be promoted, rather than relying on health promotion and awareness. These interventions could include loan or credit scheme support, and also the award of safety certificates to farmers and vendors who comply with prescribed risk reduction measures. The use of social constructs or emotional drivers could also be implemented, together with good agricultural practices at farms and hygienic practices at markets and kitchens. Above all, interventions are likely to be successful if they are implemented in a participatory manner to involve government, at-risk groups and other major stakeholders.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Prince Antwi-Agyei
Principal Supervisor	Dr Jeroen Ensink
Thesis Title	Wastewater use in urban agriculture: an exposure and risk assessment in Accra

<u>If the Research Paper has previously been published please complete Section B, if not please move to</u> <u>Section C</u>

SECTION B – Paper already published

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Where is the work intended to be published?	**Water Research**
Please list the paper's authors in the intended authorship order:	Prince Antwi-Agyei, Jeroen Ensink, and Paul Hunter
Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	My supervisor and I designed the study. I developed all data collection tools and collected field data. I was guided on the QMRA modelling, and was the lead author for the manuscript.

Student Signature: ____________________ **Date:** ______________________

Supervisor Signature: __________________ **Date:** _____________________

Chapter 5: A quantitative microbial risk assessment of norovirus risk to consumers of salad produce irrigated with wastewater in Accra, Ghana.

Photo 5.1: A street food vendor using wastewater to wash salad crops at wastewater irrigated farm in Accra, Ghana

Abstract

Where wastewater is used for agriculture, the WHO has developed guidelines for the safe use of wastewater in agriculture in order to protect human health. The current guidelines use a Quantitative Microbial Risk Assessment (QMRA) approach to estimate and manage risk arising from pathogens, though the reliability of risk estimates from these models are often questioned due to a lack of primary data. The current study attempted to improve the reliability of estimates from the WHO QMRA model by using field-based data collected from Accra, Ghana to estimate the risk to consumers of wastewater irrigated produce. The main aim was to assess how the use of an exposure field-based data influenced the estimated risk compared to the use of a predominantly secondary-based data as often used in indicator-based QMRA models. The study employed the pathogen water model, together with the 10,000 Monte Carlo simulations to estimate the risk to consumers.

Results from the study showed that there were no quantifiable norovirus concentrations on produce from farms and markets, and also in prepared salad sold by street food vendors, though a mean concentration of 6.48 Log genes copies/100 ml was found for all positive samples of norovirus GI and GII in irrigation water. The daily and annual norovirus infection risks for consuming contaminated salad food at home, and at street food stalls were 5.19×10^{-5} per person per day (pppd) and 1.89×10^{-2} pppy respectively, and were found to be higher than the WHO acceptable guidelines. Although the use of pathogen concentrations to estimate the risk makes the current model better in predicting risk than the use of indicator QMRA models, both models appear to underestimate consumers' risk since they ignore other sources of contamination at the farms, and also at the points of consumption. Further studies on consumer surveys, behaviour exposure assessment, and estimation of virus and other pathogen concentrations in environmental and food samples are needed to reduce uncertainties and to help refine QMRA risk estimates.

Keywords: Quantitative microbial risk assessment, norovirus, wastewater reuse, urban agriculture, Ghana.

5.1 Introduction

The use of wastewater in agriculture is seen by many as a way to preserve good quality water, and to overcome water scarcity. However, in many low and middle income countries in the absence of wastewater treatment, wastewater is often used untreated, or partially treated, exposing farmers, crop handlers, and consumers to a variety of health risks [31]. In order to protect human health, the World Health Organisation (WHO) has developed guidelines for the safe use of wastewater in agriculture [3]. The WHO guidelines have undergone several revisions but the current version [3] represented a departure from the first two editions which relied on epidemiology and microbial water quality standards [26, 27].

The current guidelines use a Quantitative Microbial Risk Assessment (QMRA) approach to estimate and manage health risk arising from pathogens. This new approach arose due to debates on the level of acceptable water quality for irrigation, mainly as a result of a lack of good quality studies to support the epidemiological approach advocated by the WHO [31]. The QMRA is considered a more sensitive tool to estimate risk that would otherwise be more costly, and difficult to measure by epidemiological investigations [3]. In the guidelines, the QMRA is combined with 10,000-trial Monte Carlo risk simulations to estimate risk, and then determine the pathogen reductions needed to achieve an acceptable disease burden of $\leq 10^{-6}$ DALY loss per person per year (pppy) [3]. Although useful, the reliability of health estimates from the QMRA models depends on the availability, and quality of its input data (for example, the occurrence, persistence and human dose-response of pathogens in the environment), which in the case of wastewater use in agriculture are often unavailable, but extrapolated from different datasets. This data originates mostly from high income countries, and hence prone to various forms of bias when applied to low, or middle income country settings, with very different incidences of faecal-oral diseases and environmental conditions, which as a consequence may result in significant over or underestimation of health risks [22, 186].

In an attempt to improve the validity, and reliability of estimates from the WHO QMRA model, especially for low and middle-income countries, this study attempted to estimate pathogen infection risks for consumers of wastewater irrigated produce by using field-based data collected from Accra, Ghana. The main aim was to assess how the use of an exposure field-based data influenced the estimated risk compared to the use of a predominantly secondary-based data as often used in indicator-based QMRA models [3, 102]. In addition, the study uses a different approach including the direct use of pathogens in wastewater to estimate the risk to consumers and compares this risk to the result from the WHO promoted QMRA model, which often relies on indicator-pathogen ratios.

5.2 Methods

The QMRA assessment presented here follows the risk assessment paradigm for human effects that covers hazard identification, exposure assessment, dose-response assessment and risk characterisation [22]. The QMRA principles together with Monte Carlo sampling method (QMRA-MC) were employed in @risk software (risk and decision analysis software) to estimate the risk of norovirus infection to consumers of wastewater irrigated produce in Accra, Ghana. Soil, wastewater and crop quality data were collected from wastewater-irrigated fields, markets and kitchens, and were accompanied by an exposure assessment of risk factors at each domain. The details of methods used to collect behavioural and microbiological exposure data have been presented elsewhere (Chapters 2, 3 & 4). Risk models for dose response assessments were based on peer-reviewed publications, but effort was made to adopt less complicated models which have been used on human challenge study data, and which was purposely designed to create dose-response models [57, 187]. All fieldwork for observations, interviews, environmental and food sampling and laboratory analysis was done from October to December 2012 in the dry season, and from June to August 2013 in the rainy season.

5.2.1 Hazard Identification

The hazard identification step describes the acute and possible chronic human health effects associated with the hazards. In this study microbial hazards considered were: human norovirus and adenovirus in food and environmental samples. Both viruses are transmitted through the faecal-oral route, and were selected because of their high burden of disease. Viruses, but especially norovirus, have been ranked as a major cause of food borne diseases, and have been identified as major agents of viral gastroenteritis in both children and adults [22]. Norovirus is highly infectious, has a very short incubation period, and is the leading cause of gastroenteritis, and accounts for almost a fifth (18%) of all cases of mild and acute gastroenteritis in all age groups worldwide [22, 188]. Norovirus can also persist for a longer time in the environment especially in water, has high shedding rates, and is highly resistant to treatment and many disinfectants [87, 122, 189]. In the United States alone, norovirus causes an estimated 5.5 million cases each year (58% of all foodborne illnesses), with 14,550 hospitalizations (26% of all foodborne related hospitalizations) and 149 deaths [131].

5.2.2 Exposure Assessment

The exposure assessment determines the size and nature of the population exposed to the hazard, the route, amount or concentration, the duration and the distribution of the hazard [22]. The size of consumers of salad crops was determined from published literature while the frequency and quantity of salad consumption were determined from questionnaire-based consumer surveys and laboratory experiments. From the consumer survey (Chapter 4), 51% of customers who bought produce at the markets reported to consume salad at home for an average of 2.8 times/week (146 d/y), while 89% consumed salad at street kitchens (fast food, locally known as “check-check”) for 4.3 times/week (224 d/y, Table 5.1). The amount of salads consumed daily at home was estimated using the average weight of lettuce bought at markets, and the number of lettuce used to prepare a salad meal for an average family size of 4. The quantity of salad consumed at street food vendor level was based on a national consumer survey by the International Water Management Institute in Ghana [94]. Based on these, the estimated quantity of lettuce consumed as salad at home was 51 g/meal, while the corresponding quantity of lettuce consumed at street vendor stalls was 13 g/meal. The number of consumers of salad food, especially from the fast food industry in Accra has been estimated to range between 130,000 – 300,000 [48, 94]. The study focused on adult consumers (range 11 – 54 years, Chapter 4) since children under five rarely consume street food served with salad.

Lettuce was chosen as the model crop due to its popularity as a salad vegetable, the fact that it is consumed uncooked, and for being the commonest crop cultivated by almost all wastewater farmers in Accra. Lettuce is grown all year round, and on average farmers have 9 cropping seasons for lettuce, compared to 3 for spring onion, and 1 for cabbage. The type, size and shape of lettuce leafs also enables it to retain large volumes of water, and hence has a higher potential to transfer pathogens from irrigation water compared to for example, tomatoes, cabbage and spring-onions.

The amount of irrigation water retained on produce was experimentally determined at the laboratory. Samples of ready to harvest lettuce (N = 52) were collected from wastewater-irrigated fields, and their weights measured. A total of 500 ml of water was then added to each head of lettuce in a bowl, and excess water was allowed to drain within 5 minutes. The combined weight of water and produce was recorded, and the amount of water left on each head was then estimated as the difference in weight of produce before and after. Urban agriculture farmers in Accra predominantly use watering cans for irrigation of vegetables, and do not practice irrigation cessation, though in the rainy season, the average withholding period between the last day of irrigation and harvest was 2 days (56 hours, Chapter 2). At markets, lettuce was stored for an average time of 10 hours but for a maximum of 48 hours before sales (Chapter 3). A maximum period of 3 days was used in the model to represent the time between last irrigation of produce, and consumption of that produce as salad (Table 5.1).

Table 5.1: Model input parameters and distributions

Input parameter	Unit	Distribution type (values)	Source/references
Probability of positive sample		13/79, uniform (0, 1)	Field data
Probability of negative sample		66/79	Field data
Norovirus concentration for positive samples (GI + GII)	Log Gec*/100ml	Normal (6.48, 0.66)	Field data
Amount of water left on crop after irrigation	ml/g	Normal (0.34, 0.10)	Field data
In-field virus kinetic decay	Day^{-1}	Normal (1.07, 0.07) – truncated at zero ~ 1.07	[190, 191]
Irrigation cessation by farmers (Rainy season, n = 80)	≤ 1 day 1 – 2 days ≥ 2 days	32 (40%) 13 (16%) 35 (44%)	Field data
Withholding period (length of environmental exposure)	Days	Uniform (0.2, 3.0)	Field data
Proportion of irrigation water remaining on crop after produce washing	Proportion	0.9 (deterministic)	Assumption based on expert opinion
Probability of consuming street vended salad	Days	224/365, uniform (0, 1)	Field data
Probability of consuming salad at home	Days	146/365, uniform (0, 1)	Field data
Quantity of salad consumed at home	g/day	51 (point estimate)	Field data
Quantity of street vended salad consumed	g/day	13 (point estimate)	Field data
P = fraction of susceptible population	Proportion	0.722	[187]
Mean aggregate size	Genome copies	1,106	[187]
Method of treatment by domestic consumers, %	%	Clean water = 28 Salt water = 58	Field data

(N = 159)		Vinegar = 14	
Method of treatment by street food vendors	%	Clean water = 21 Salt water = 14 Vinegar = 65	Field data

* *Genomic copies*

The estimated risk was compared to the worst case scenario (model) where people bought directly from farms without any irrigation cessation, and hence no pathogen die-off due to in-field kinetic decay.

Virus presence/absence, and inhibition in water, soil and on produce was determined using Quantifast Pathogen IC RT-PCR and PCR kits while concentrations of Norovirus and adenovirus were determined using Qiagen OneStep kits [108]. All microbial concentrations were modelled using $\log_{10}$ normal probability distributions [22, 192]. Under the exposure assessment, the following risk pathways (exposure models or scenarios) were considered for consumers' risk. These models/scenarios were considered as salad was consumed at farms (farmers use it as an accompaniment to other food during work), markets (market vendors), street kitchens (street food consumers), and at homes (households):

a) Consumption risk based on irrigation water quality (water model)
b) Consumption risk based on produce quality at farm (farm model)
c) Consumption risk based on produce quality at markets (market/home model)
d) Consumption risk based on microbial quality of prepared salad from street kitchens (street food model).

The daily dose of norovirus (d_w) likely to be ingested by consumers was estimated from the Equation below [193]:

Water model: $$d_w = \frac{\mu_w m V w_r}{kt} \quad (1)$$

Where μ_w is the concentration of norovirus in irrigation water (genome copies/ml), m is the per capita mean quantity of salad consumption at home, or at street kitchen or both (g/meal/person/day), V is the amount of irrigation water left on produce after irrigation (ml/g), w_r is the $\log_{10}$ reduction ($\log_{10}$ units) of pathogens after washing of produce prior to salad consumption. The rest were k, the in-field virus kinetic decay constant (day^{-1}) and t, the time between the last irrigation application and consumption (days). The virus decay constant was based on a similar study in Australia which adopted the kinetic decay constant for

Bacteroides fragilis bacteriophage B40-8 in their model, due to the absence of decay data for norovirus. Bacteroides fragilis bacteriophage B40-8 is more resistant relative to other pathogens to decay in environmental conditions and therefore is a conservative model for human enteric viruses [190, 191, 194].

It was assumed that pathogens in the quantity of water left on produce remained on the produce even after the water had evaporated [63]. Based on a previous study [140], pathogen reduction of 2 log units arising from produce washing, or disinfection was also included in the model (Equation 1) as washing of produce was a common practice among vendors and consumers of salad produce (Chapters 3 & 4). All general input parameters and their probability distributions are presented in Table 5.1.

5.2.3 Dose - Response Assessment

The dose response process provides a quantitative estimate of the risk of response (infection, illness/disease or death) with respect to the dose of pathogen. The Fractional-Poisson model which is a modified version of the Beta-poisson model (β-Poisson) was used to model the infection risk of norovirus [187]. Building upon the dose-response model from Teunis *et al.*, [57], the fractional-Poisson model considered two parameters, host susceptibility which it characterizes as either susceptible, or immune, and a second parameter which accounts for virus aggregation [187]. The pathogen infection risk is then estimated as the product of non-zero exposure (i.e. probability that at least one virion is ingested) and the fraction of susceptible hosts; i.e. the single infection of the pathogen infection is given by the Equation below [187]:

$$P_{\inf}\left(Dose(d), P\right) = P \times \left(1 - e^{-Dose(d)/\mu}\right) \tag{2}$$

Where P is the fraction (proportion) of susceptible subjects, d is the daily dose of norovirus ingestion (genome copies/meal/person/day) by consumers, *e* is Euler's number or constant (2.718282) and μ is the mean aggregate size (or number of viruses (genome copies) per aggregate). Based on a maximum likelihood estimation, and the Akaike information criterion (AIC) for the fractional-Poisson model, and the Beta-Poisson model, a mean aggregate size of 1,106, and the fraction of susceptible subjects of 0.722 corresponding to the fractional-Poisson model were considered as more appropriate fit for use in the risk model for this study (Table 5.1) [187].

5.2.4 Risk Characterization

The risk characterization stage integrates information from the first three steps to estimate the magnitude of the public health problem, and to evaluate noteworthy conclusions, variability and uncertainty. The following exposure pathways and risk scenarios were considered:

i. Risk of pathogen infection among household and street-food consumers of salad
ii. Comparison of risk estimates between the use of actual pathogens, and the use of indicator to pathogen ratios.

In the first scenario (i), actual concentrations of pathogens (norovirus) in irrigation water was used to estimate the pathogen infection risk among consumers of wastewater irrigated produce at homes or at the street food vending sites. This was done due to the absence of norovirus in street vended salad and also produce bought from markets to be used in the house. It was also assumed that the quantity of pathogens in irrigation water left on produce would remain even after the water had evaporated markets, homes and at street kitchens. The risk estimate from scenario one was then compared to the use of indicator pathogen ratios from literature (i.e. no direct use of pathogen concentrations) in the second scenario (ii).

The annual risk of pathogen infection is given by: $P_{i(A)}(d) = 1 - [1 - P_I(d)]^n$ (3)

where $P_{i(A)}(d)$ is the annual risk of infection in an individual from n exposures per year to the single pathogen dose d, and $P_I(d)$ is the risk of infection in an individual exposed to (here: following ingestion of) a single pathogen dose d.

5.2.5 Analysis

Data analysis was done using STATA 12 (StataCorp LP, College Station, USA) and @Risk 6 add-in to Microsoft Excel 2013 (Palisade Corporation, NY-USA). @Risk was used to model the risk of infection to consumers by combining pathogen concentrations, and other exposure variables with the 10,000 Monte-Carlo Simulations. The median pathogen infection (50% value), 90-percentile infection (90% value), and the annual pathogen infection (PI Annual) were reported. In the water model, concentrations of norovirus (NV-GI and NV-GII) were left censored (most concentrations were too little to count, or were below the minimum detection threshold), and so in order to increase statistical precision (reduce confidence interval by increasing data points), consumers' exposure probability to hazard was modelled to account for both positive, and negative samples for the two viral genome groups (GI &

GII) by randomly sampling from the distribution using "what if" scenarios. A sample was considered positive if it was positive for either NV-GI, or NV-GII or both, otherwise negative. This also means that a consumer could ingest either one of the genome groups of norovirus, or both when exposed. Similarly, the per capita consumption of salad food was modelled as a probability distribution of consuming salad either at home, or at street food kitchens, or both. Model variability, randomness, and uncertainty of parameters were accounted for using appropriate probability distributions (e.g. uniform and lognormal distributions), and the 10,000 MC simulations. Statistical sensitivity analysis was done using the Spearman Rank order correlation after the 10,000 iterations of input parameters to assess the robustness of model results and more importantly to assess the uncertainty relationship between input parameters and the daily probability of infection. All input parameters (Equation 2 and Table 5.3) that could influence the risk of pathogen infection including pathogen concentrations, dose-response parameters and dose of pathogens exposed in certain quantities of food consumed at homes and on the street were included in the sensitivity analysis. The assumption was that each of these uncertain variables was modelled with a single @RISK distribution and that each distribution was considered separately in the model.

5.3 Results

5.3.1 Health risk to consumers

Almost 17% (N = 79) of irrigation water samples were positive for either NV-GI or NV-GII, or both. Mean concentration of all positive samples of norovirus GI and GII in irrigation water was 6.48 Log genes copies/100ml (Table 5.1). The average amount of irrigation water left on lettuce was also estimated to be 34 ml/100 g (range, 14 – 51 ml/100 g, N = 52).

The study employed only the water model to estimate the risk to consumers since there were no quantifiable norovirus concentrations on produce at farms and markets, and also in prepared salad sold at street food stalls. Overall, the median daily norovirus infection risk for consuming contaminated salad food at home, and at street food stalls was 5.19×10^{-5} per person per day (pppd) (90% CI = 7.94×10^{-6}, 7.24×10^{-1}), with the majority of the probability distribution (84%) lying between 3.16×10^{-6} and 7.94×10^{-4}, and about 15% of the distribution falling within the maximum risk (Figure 5.1). The median daily infection risk for consuming salad at street kitchens, or at home was similar (1.84×10^{-5} vs. 7.23×10^{-5}). The median annual norovirus infection risk for consuming street vended salad, and salad at home was 1.89 x 10^{-2} pppy (90% CI = 2.82 x 10^{-3}, 2.63 x 10^{2}, Figure 5.2). Both the median daily (8.91 x 10^{-5}) and annual risk (3.31 x 10^{-2}) to consumers who bought produce directly at farms where produce was irrigated on the same day of harvest was not different from those who consumed salad after a waiting time of up to 3 days between last irrigation and consumption. Sensitivity analysis showed that uncertainties in the salad consumption at home, the probability of detecting positive/quantifiable samples, and the duration between last irrigation and consumption of produce were the most influential factors, but were all negatively correlated with the probability of infection with Spearman correlation coefficient ranging from -0.40 to -0.45 (Table 5.2). Uncertainties in the amount of irrigation water left on produce after irrigation, and the salad consumption patterns at street kitchens were positively correlated to the infection risk but the effect was weak. Uncertainties in the in-field kinetic decay, and the average norovirus concentration for positive samples for the two genome groups had non-significant influence on the variation of the infection.

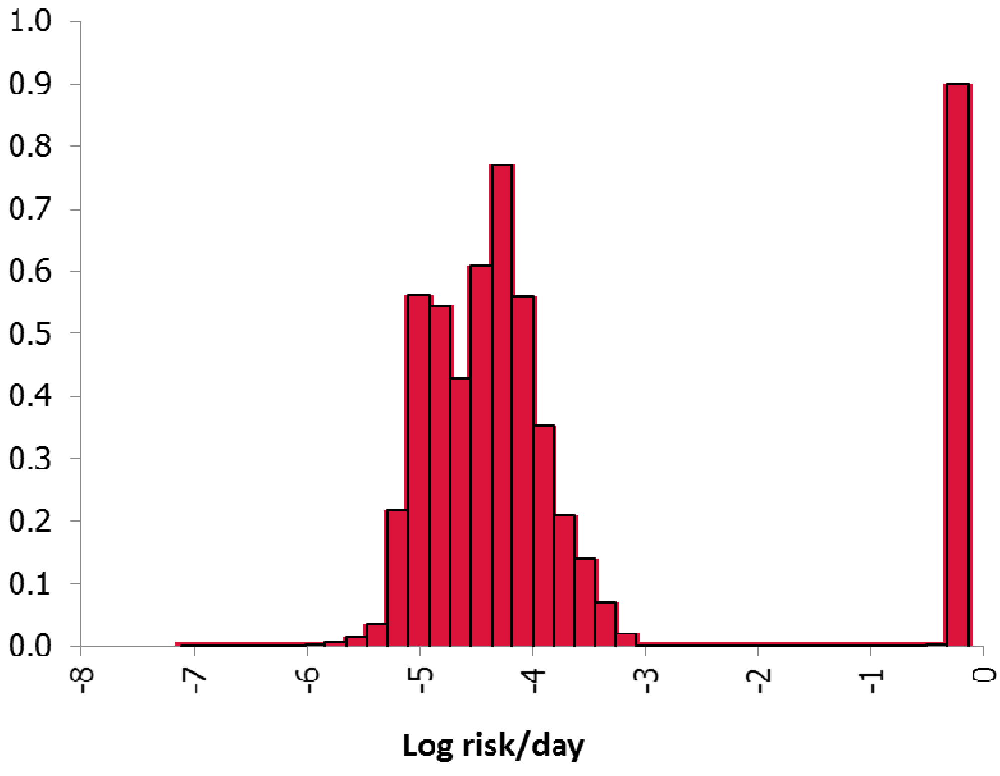

Figure 5.1: Daily probability risk of infection to consumers of salad

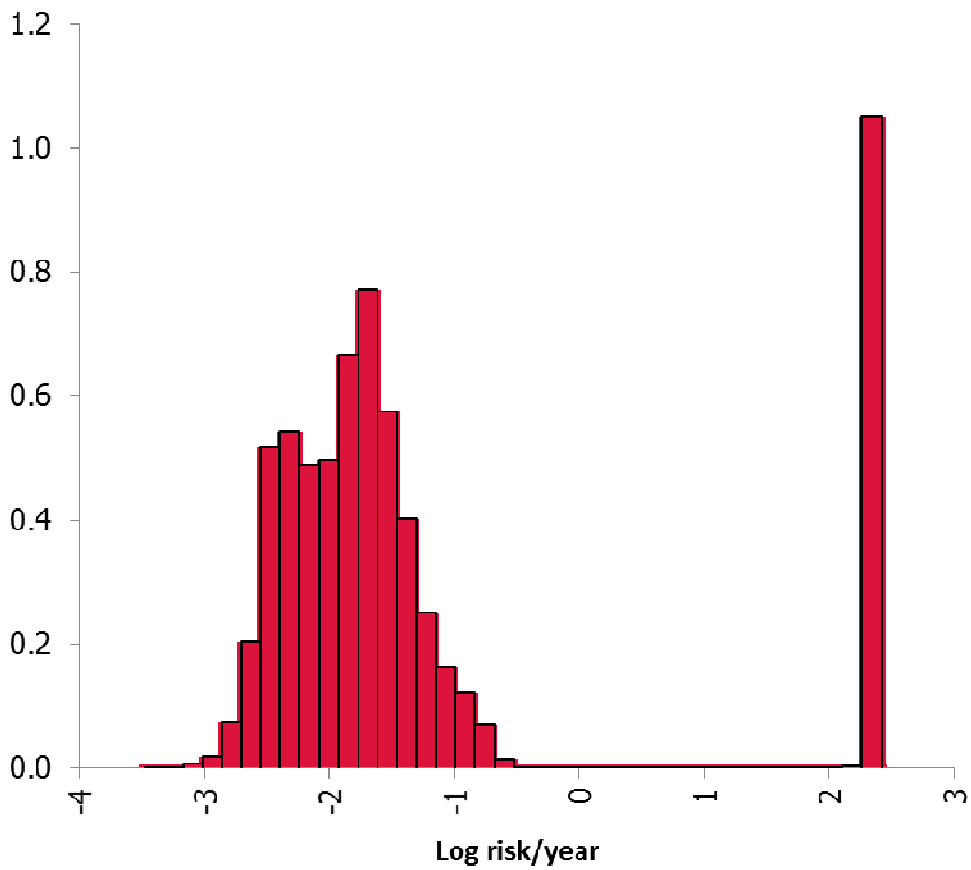

Figure 5.2: Annual probability risk of infection to consumers of salad

Table 5.2: Sensitivity of the probability of norovirus infection to variation in input random variables

Input parameter	**Spearman rank order correlation coefficients**
Quantity of salad consumed at home	-0.44
Probability of positive sample	-0.42
Time between last irrigation and consumption	-0.40
Water left on produce	0.21
Amount of salad consumed at street kitchen	0.15
In-field virus kinetic decay	-0.04
Distribution of positive samples (count if positive)	0.00

5.4 Discussion

The study found that consumers' risk exceeded the WHO guidelines, however the use of indicator QMRA models, or the pathogen-based water models could all underestimate consumers' risk.

5.4.1 Comparison of consumer risks from pathogen-based water model and indicator-based QMRA models

The findings from this study showed that the risk of consuming salad produce irrigated with wastewater exceeded the maximum tolerable risk (TR) of norovirus infection (10^{-3}), which corresponds to a DALY loss of 10^{-6} pppy [3, 195]. The estimated risk from this study could not be compared with similar studies due to the limited number of studies that have measured, and used direct norovirus concentrations in irrigation water to estimate consumer health risk. In contrast, most studies have relied on indicator based QMRA models to estimate norovirus risk, predominantly using a given ratio between concentrations of *E. coli* or thermotolerant coliforms, and norovirus or other enteric viruses [66, 195]. Although the use of indicator based QMRA models continue to be the preferred choice due to the lack of data, and the fact that *E. coli* is a lot cheaper and easier to measure, the approach is problematic since it ignores several other factors such as seasonality, transport characteristics of microbes and other environmental factors (humidity, UV light etc.) which could all affect the correlation between the indicators and the pathogens [24, 61]. In an effort to overcome the above problem and assess the reliability of estimates from the WHO indicator QMRA model, the current study used actual pathogen concentrations in irrigation water to estimate consumer risk. The study found that the median annual norovirus infection risk from the pathogen-based water model was one order of magnitude lower than the infection risk from the WHO QMRA model used in the farm to fork study (Chapter 3) (1.89×10^{-2} vs. 1.4×10^{-1}). There are several reasons that could account for the difference in risk between the two models. The WHO QMRA model used in the farm to fork study adopted the widely used relationship of $1:10^5$ between *E. coli* and viruses, which was based on one study in Northeast Brazil that reported concentrations of faecal coliforms and enteroviruses [128]. A recent study in Accra, however, found an average of one norovirus GII to $10^{3.2}$ *E. coli* or $10^{4.8}$ thermotolerant coliforms, from its quantifiable irrigation water samples [60], and hence larger than the ratio found in the Brazil study. In the current study, the ratio of means between norovirus and *E. coli* was also estimated as 2.1×10^{-2} (or $1:10^{1.7}$) for irrigation water samples that were analysed for both *E. coli* and norovirus (N=67). Although most of these studies assumed linear relationships between these microbes, which could even be computationally incorrect, the variations in the ratios suggest that there is inadequate evidence to support one particular relationship. The other possible reason for the difference in risk estimates could be attributed to the fact that the WHO model used the Beta-poisson dose-response model on the direct norovirus dataset from Teunis *et al*. [57], unlike the current study, which employed the fractional-poisson dose-response model [187].

Other indicator-based QMRA models have also provided varied results to the pathogen water model used in the current study. However, the estimated risks from those models were also higher than the tolerable infection risk for norovirus/rotavirus. For example, Mara and Sleigh [195] found that consumers were at risk of norovirus infection (10^{-2} pppy) if they consumed 100 g of salad crops irrigated with wastewater of at least 10^2 to 10^3 *E. coli*/100 ml quality, for every two days. A study in Ghana, also used indicator QMRA water model and found that the risk of rotavirus infection (2.3×10^{-3}) was higher than the WHO tolerable risk for rotavirus diarrhea in developing countries (7.7×10^{-4}) [66]. Similar findings were found in the United States where the mean annual risks (10^{-3} to 10^{-1}) of enteric viruses from consuming various salad crops exceeded the benchmark risk of $\leq 10^{-4}$ even when wastewater (pre-treated) irrigation was ceased one day before harvest [58]. To avoid the possible complications of the indicator pathogen relationship, some studies have used alternative approaches to estimate virus concentrations in wastewater. For example, a study in Australia used the human faecal shedding rates method to estimate norovirus concentrations in raw wastewater ($6.03\ 10^7$ virus/L). The study found that the estimated pathogen risk ($7.95\ 10^{-5}$ to $2.34\ 10^{-3}$ DALY pppy) also exceeded the WHO acceptable limits (10^{-6} DALY/person/year) even after waste stabilisation treatment [196]. From the aforementioned results and discussions, it is increasingly becoming clear that the use of indicator pathogen relationships, and the water models, are likely to result in a higher risk than the tolerable thresholds, though other input parameters could affect the estimated risk. One way of overcoming this challenge would be to conduct further and more microbial assessments to establish a better correlation or relationship between pathogens and indicators, or where resources are available to use direct concentrations of pathogens to model pathogen risk of infection.

5.4.2 Strengths and weaknesses of model assumptions

Ideally, the best measure for consumer risk should be based on actual pathogen concentrations isolated from ready-to-eat food at the points of consumption (street food model or market/home model). Although intended, this study was unable to use any of these models since there were no positive samples, or quantifiable norovirus concentrations on produce at farms, markets, or in street food (Chapter 3). Technically therefore, this study would argue that the consumption of salad produce collected from farms, markets and at street food kitchens in Accra is safe and poses no risk of norovirus infection since no virus concentrations were found on these samples. However, the estimation of viruses in food samples such as salads, or even in wastewater faces a number of challenges such as: cost, poor viral detection efficiency, and no available cell culture to determine infectivity, and hence the limited number of studies that have reported direct viral concentrations in these samples [115]. Additionally, further studies within the study area would be required before the "no detection" of norovirus in food samples can be confirmed, especially when produce and salad samples were highly contaminated with *E. coli* (Chapter 3); though the presence of *E. coli*, or indicators is no guarantee of the presence of viruses [61, 62]. Discounting the "no detection" of norovirus on produce or in prepared food, the current study employed the water model but used actual pathogen concentrations found in irrigation water, instead of the indicator pathogen ratio to predict consumer risk at street kitchens and at homes. A similar study in Kumasi also predicted consumers' risk using various exposure models and found the street food model as the "closest" estimate of consumer risk. The study further found that the water model underestimated consumer risk consistently, when compared to the market produce and street food models [193]. Unlike the current study that used direct virus concentrations in the water model, the Kumasi study relied on ratios between *E. coli* and virus concentrations in wastewater, though in this case it applied virus percent recoveries to help account for uncertainties in the assumed ratios. However, results from the two studies seem to suggest that the water model underestimates consumers' risk since it ignores other potential contaminants of produce at the farm such as soil quality and the use of animal manure, and also post-harvest contamination of produce or prepared salad. A farm-to-fork study (Chapter 3) that explored different exposure models also found that the street food model resulted in the highest risk to consumers, though the risk from the water model was higher than the produce models at farms and markets.

Another strength of the current model is that, consumers' risk was estimated by considering the probability of exposure to both negative (transformed) and positive samples in order to avoid overestimation of the risk if only pathogen concentrations of positive samples were used. Most of the input data for the current study originated from the same dataset, and from the same study area (Table 5.1), and hence less prone to uncertainties in the risk estimates compared to the use of models dominated by input parameters from different dataset, and also from different geographical areas with different exposure settings. The fractional poisson model adopted for the dose-response model was computationally simple, and has been validated to best describe data from the norovirus human challenge from which it was applied to [187]. The model uses lesser parameters compared to the beta-poisson which was originally used to describe the norovirus human challenge study data [57, 187].

5.4.3 Sensitivity analysis

In most previous studies [196], uncertainties in the consumption rate and virus concentrations were found to have the most or significant impact on the variation in estimates of the risk of infection/disease. In the current study, uncertainties in the amount of salad consumed at home, the detection of positive samples, or quantifiable concentrations, and the time between last irrigation and produce consumption were the most influential factors affecting the variation in the pathogen risk. The variance in norovirus detection, and quantification could have been contributed to the several RNA extracted samples (about two-thirds) that showed inhibition of the internal control as well as increased inconsistency between duplicates from the same sample. Additionally, the lower limit of detection (LLOD) for norovirus was approximately 5,000 virus particle units, and the fact that norovirus in particular was detected as very low rates (left-skewed distribution), could have contributed to some potential biases in the distribution. Samples with no detected virus were, however, transformed by 0.5 times the LLOD to account for potential biases in distribution. Among other things, this could also imply that improvement in virus detection limits or measurement techniques and actual consumer based survey at the household level could improve the risk estimates from QMRA models.

5.5 Conclusion

The results from this study showed that consumers were at risk of norovirus infection since the estimated risk was higher than the acceptable limits recommended by the WHO. The use of actual pathogen concentrations to estimate the risk makes the current model better in predicting risk than the use of indicator QMRA models. However, the pathogen water model appears to underestimate consumers' risk since it ignores other sources of contamination at the farms, and also at the points of consumption. Further studies on consumer surveys, risk behaviour exposure assessment, and estimation of virus and other pathogen concentrations in environmental and food samples are needed to reduce uncertainties and help refine QMRA risk estimates.

Chapter 6: Discussion, conclusions and recommendations

Photo 6.1: Market salespersons transporting salad vegetables to markets after harvest at wastewater irrigated farms.

6.1 Summary of study findings

The overall aim of this thesis was to test the appropriateness, and to strengthen where possible, the current QMRA and multi-barrier approach advocated by the WHO guidelines for the safe use of wastewater in agriculture. In order to do this, four main objectives were set out, and the key findings are discussed in this Chapter.

Chapter 2 presented an exposure assessment that identified key exposure pathways associated with the transmission of faecal pathogens in farmers using wastewater for irrigation. The study found that, although irrigation water was significantly more contaminated than farm soil, exposure to soil was the key risk pathway when assessing farmers' faecal oral disease transmission of pathogens. Hand to mouth contact events were common, but were only associated with soil related farming activities, and not during irrigation. Farmers' risks for norovirus infection were found to have exceeded WHO guidelines, though the study was unable to determine whether the estimated risk was an overestimation or underestimation due to limitations of the QMRA model.

In Chapter 3, a cross-sectional study was presented that aimed to identify potential risk factors of salad produce from farm to fork. The study found high levels of faecal contamination on produce at all entry points along the food chain, though the highest levels were found at the street vending sites. At farm level, the impact of soil contamination on produce quality was found to be more pronounced than the effect attributed to irrigation water. The study could not establish whether the use of wastewater had any significant influence on produce quality at markets and kitchens, but it was clear that post-harvest practices had a significant impact on the quality of salad produce especially at street kitchens. The study found the faecal contamination levels found on salad food exceeded the WHO guidelines for the safe use of wastewater in agriculture.

Chapter 4 of the thesis presented the results from a risk perception study that aimed to assess participants' awareness and knowledge of wastewater irrigation practices, associated health risks, and the adoption of health protective measures. Overall, the study found that awareness of how produce might be irrigated was low, especially among consumers, while knowledge of wastewater associated health risks alone was not sufficient to adopt health reduction measures; nor to influence consumers when buying produce, or prepared salad. In contrast, knowledge of the actual sources of hazards especially on the type and use of wastewater was more likely to influence consumers to buy or consume salad produce and possibly lead to the adoption of risk reduction measures.

The last chapter of the results section (Chapter 5) presented a quantitative microbial risk analysis (QMRA) that aimed to estimate the health risk to consumers using actual pathogens concentrations and contact patterns. The study found that consumers of wastewater-irrigated produce were at risk of infection, since the estimated risk exceeded the WHO recommended level. Based on the available data, however, the results from the model were inconclusive to predict an actual risk to consumers.

6.2 Wastewater use and produce quality

Although the 2006 WHO guidelines technically do not rely on water quality thresholds, the current WHO QMRA approach employed to estimate risks among wastewater workers and consumers of irrigated produce implicitly relies on the quality of wastewater. Based on the study design, and the available data, this study was unable to establish evidence on the impact of wastewater irrigation on the microbial quality of produce beyond the farm level, though it was clear that the use of wastewater had a significant influence on produce quality at the farm level. At farm level, soil contamination was found to better explain levels of produce contamination than wastewater (unit increase of 0.59 Log *E. coli*/g produce vs. 0.14 Log *E. coli*/g produce), though the contamination of soil was also influenced by wastewater and the use of chicken manure. The seasonal variations, with higher *E. coli* concentrations found on farm produce during the dry season, and the higher use of poultry manure in this season would argue, that the use of chicken manure might be a greater risk than irrigation water. Pathogenic *E. coli* O157:H7 is found to survive in vegetable farm soil treated with animal manure for up to 6 months, significantly longer than in soil without animal manure [197]. The above findings therefore suggest that soil and manure management should be considered as key sources of produce contamination at the farm level, and must be captured in the updated WHO guidelines. This study can provide no further evidence on the role of: sunshine, temperature and relative humidity on the survival of *E. coli* on crops, since produce samples in both seasons were collected within the same time period, and also under similar field conditions. They are, however, likely to play a role, and consideration should be given to incorporate these within the QMRA model.

Only two field-trial studies have provided evidence on the impact of wastewater use on produce quality at local markets, as a result the relative importance of wastewater irrigation in disease transmission across the different domains remains a contentious issue. This becomes more important especially as both studies provided contrasting findings. Work in Pakistan found the use of wastewater to have little impact on produce quality at local markets, with poor post-harvest handling practices at the markets the key risk [65], while work in Ghana found that the farm domain contamination was the primary source of produce contamination [48]. In Pakistan the lower levels of produce contamination at the farms were attributed to irrigation methods that prevented direct contact with produce, and environmental conditions that contributed to rapid pathogen die-off [65]. Produce contamination at farms in Ghana was attributed to the use of improperly composted poultry manure to manage soil fertility and the use of watering cans which applied water directly on the produce and caused soil to splash on produce. The concentrations of *E. coli* on produce following harvest can be expected to reduce with time, and as a result of environmental conditions. For example, a field trial in Nova Scotia, Canada, to determine the survival of *E. coli* on market-ready spinach after spray inoculation showed a reduction of 3-5 log units after 72 h [198]. The reduction of *E. coli* with time across domains was not shown in this study, especially during the rainy season and could suggest that post-harvest handling and practices did influence contamination levels at markets and kitchens. In addition, unlike the study in Pakistan that collected samples at market from the same lot as those collected at farms, this study sampled general market produce and it is therefore unclear what the proportion was of produce at markets and kitchens that was actually wastewater irrigated. Despite this limitation, the findings from this study suggest a clear need to prioritise risk reduction interventions at markets and kitchens as most consumers buy produce directly from these domains, and not at the farm level. This also means that the WHO QMRA model for assessing consumption risk should use concentrations of microorganisms or pathogens on produce at the points of consumption, and not concentrations potentially attributed to wastewater. This is particularly important since time and environmental conditions are likely to reduce pathogen concentrations, something for which the model currently makes no allowance for.

6.3 QMRA and the WHO guidelines

The use of the QMRA approach in the guidelines was meant to overcome the limitations of the microbiological criteria by accounting for ingestion dose, population exposed to hazard, and the frequency of exposure to hazard [102]. However, the challenge with QMRA is that most of the input data required, are often unavailable, and hence there is a degree of uncertainty around its risk estimates. In order to improve the reliability of QMRA estimates, the current study was designed to provide primary input data, on pathogen concentrations in soil, water and on produce, farmers' exposure frequency with contaminated soil, direct hand-to-mouth events and the key farming activities that expose farmers to faecal pathogens, and also salad consumption patterns.

6.3.1 Improving QMRA estimates for farmers' occupational risk

This study found contact with soil to be the main risk for farmers, with an average of 10 hand to mouth contacts per day during tilling of the soil, and nil during irrigating. This would confirm that the WHO QMRA model to assess risks to farmers based on accidental soil ingestion is correct. An earlier study in Accra came to a similar conclusion [66]. However, they suggested the inclusion of wastewater exposure in the QMRA model for a better assessment of farmers' risk, though their justification for this recommendation was unclear. In Ghana the use of watering cans for irrigation makes ingestion of water unlikely as their hands are holding the watering cans, though farmers could ingest wastewater through drip or spray irrigation, or just after irrigation if they fail to wash their hands with clean water and soap. This further highlights that the model might need to include an option for different irrigation techniques to accurately model risks.

The use of self-reported exposure frequency for farmers in the QMRA model could significantly overestimate the risk to farmers, since this study found that the actual time spent involved in 'risky' activities was at least 50% less than the self-reported time that farmers spend at their farms. The time farmers spend at their farms is particularly important when assessing farmers' risk transmitted via dermal contact (e.g. hookworm infection) as almost all farmers were found working bare-feet for most of the time, though this is less relevant for oral ingestion. The QMRA model assumes a range for the amount of soil potentially ingested by farmers, without considering the actual incidences of hand-to-mouth contacts which was found to be the key exposure pathway for this kind of risk. Incorporating hand-to-mouth events in the model will make it possible to determine the likely ingested dose of pathogen per hand-to-mouth event, and for all events per day. This would allow a more accurate modelling of actual ingestion over a day, and per season, and is likely to result in a more accurate prediction of disease risk.

6.3.2 Improving QMRA estimates for consumption risk

The use of indicator-pathogen ratios for risk estimations can lead to spurious results due to the complexities in these relationships, and their poor correlation with actual pathogen concentrations. Moreover, there is inadequate evidence to support the widely used ratio of $1{:}10^5$ between bacteria indicator organisms and viruses, as the results from other studies seem to suggest that this ratio could result in overestimation of the risk. In addition, the use of irrigation water quality to estimate risk among consumers of produce, rather than the use of actual microbial quality of produce at the points of consumption could also result in biased decisions on public health policies, as they do not predict actual risks. This becomes particularly important in situations where post-harvest practices have been identified as major sources of produce contamination [65], and in such instances the water model is found to underestimate consumers' risk. Even at the farm level, consumer risk will still be underestimated if the model relies only on irrigation water quality since produce quality are significantly influenced by other factors, as was shown in this study where soil quality was a better predictor of *E. coli* on produce, and also in other studies [93]. QMRA model estimates would therefore be more reliable if risks among consumers are estimated based on actual pathogen concentrations on produce, or in prepared salad at the points of consumption.

6.3.3 WHO health based target guidelines

Apart from the non-availability of key input parameters for QMRA risk modelling, another contentious parameter for risk estimation is the permissible additional disease risk arising from working in wastewater-irrigated fields, or consuming wastewater-irrigated crops. The study showed that the norovirus infection risks to farmers and consumers were both higher than the maximum tolerable risk of norovirus infection based on the WHO health based target of 10^{-6} DALY loss pppy; though for farmers the risk was within acceptable limits based on the mean soil quality. Overall, the quality of at least 51% of the soil samples collected would result in a health risk that exceeded the WHO guidelines for ingestion. For consumers, the estimated risk was 2 to 3 orders of magnitude higher than the tolerable norovirus infection limit, for the water and street food models which predicted the highest risks. The current permissible disease risk implies that farmers and consumers are unlikely to experience any norovirus infection/disease throughout their lifetime. As a result a more relaxed guideline value of 10^{-5} pppy or 10^{-4} pppy was proposed [127]. One of the reasons for the use of a relaxed DALY of either 10^{-5} pppy or 10^{-4} pppy was that the resulting norovirus/rotavirus disease risk would still be lower than the actual global incidence of diarrhoeal disease of

0.1-1 pppy in both developed and low and middle-income countries [127]. When this proposed guideline was used in this study, risks to farmers and consumers' were within acceptable or marginally outside the tolerable limits. A relaxed DALY would also result in a reduction in the cost required for wastewater treatment in case that is considered as a risk reduction measure; and hence the extra money saved could be used for other purposes including the implementation of post- harvest risk reduction interventions.

6.4 Application of the multi-barrier approach to manage health risk

6.4.1 Managing occupational risk to farmers

Similar to other studies, the findings from this study suggested that farmers' occupational risk could be minimised by controlling microbial contamination through the use of irrigation water, and poultry manure. This could be achieved by: a combination of wastewater treatment, improved hygiene, and good agricultural practices. Wastewater treatment remains the most effective way of reducing pathogens in wastewater and as a result in farm soil and is recommended whenever resources are available. Where resources are unavailable or limited, improved hygiene and human exposure control measures are required. The study found that farmers acknowledge that their farming practices could expose them to health risk, though livelihood considerations outweighed the associated risks. This also means that interventions at the farm level should focus on minimizing risks, while maintaining livelihoods. Farmers were willing to use boots if the material of the boots would cause less discomfort, often experienced as peeling of skin. Similarly, farmers preferred gloves that could prevent irritation to the skin, though one could question if the use of gloves would present a risk reduction as it may not prevent hand-to-mouth contacts. However, farmers might reduce hand-to-mouth contacts as it might be considered uncomfortable to touch their face or mouth while using the gloves. The application of wet non-dried chicken manure could be due to their lack of knowledge, on when and how to apply the manure safely. Agriculture extension officers could therefore raise more awareness, and educate farmers on the hygienic handling of manure and other forms of fertilisers. A major factor constraining farmers from investing in relatively expensive interventions on the farm was land insecurity. Local authorities and land owners could therefore collaborate with farmers through their farmer associations by entering into some forms of agreement on the land use, which could also help farmers invest more in on-farm interventions; therefore reducing health risks but at the same time ensuring their livelihood. Farmers could also protect existing dug-outs, and sedimentation ponds by incorporating steps, or platforms to help them access irrigation water without stepping their feet into it. This protection could also prevent external contaminants including runoff into the dug-outs and hence improving the quality of the irrigation water. Farmers also proposed to wash their feet with antiseptic (e.g. dettol) treated water soon after irrigation in order to protect themselves from microbiological hazards in the irrigation water. This method would, however, require further investigation as it is unclear if it would protect against hookworm infection.

6.4.2 Managing consumption risks

The findings from the risk survey showed that vendors and consumers may not necessarily adopt risk reduction measures based only on their awareness of wastewater irrigation health risks, or knowledge about diseases associated with wastewater irrigation. On the contrary, they were likely to adopt risk reduction, or health protective measures if they actually knew of the sources of water used to irrigate the vegetables they consume. The multiple barrier approach is underpinned by the involvement and commitment of major stakeholders at the various stages of the food chain, but the approach is still untested on its effectiveness. From this study, farmers knew that the sources of water used to irrigate the crops could pose health risk, but they prioritised their livelihood at the expense of the associated risk. Market vendors were not fully aware of the irrigation water sources, but those who seemed to be aware were also not ready to inform consumers since they would lose their market. Consumers are the biggest at-risk group but were also unaware of the sources of irrigation water since they were not told. The multiple barrier approach is therefore likely to work only when a balance is made on protecting public health, and also sustaining livelihoods of farmers and vendors.

Where resources are unavailable for wastewater treatment, crop restriction can be considered a suitable option, though crop restriction can have implementation challenges. Wastewater treatment, has as disadvantage, that it does not only remove pathogens, but also removes nutrients found in wastewater. This could lead to reduction in plant yields, and an increased requirement for the use of chemical fertilisers, which has been mentioned by farmers as one of the key motivations to use wastewater [199]. Crop restrictions would limit farmers in their choice of crops with many farmers mentioning that they can grow vegetables on wastewater, but not on normal irrigation water as it does not allow for frequent intervals. The salad crops grown by farmers in Ghana fetch good prices at the local markets, but the fact that they are also consumed uncooked means that they hold the highest health risk, and as a result when crop restrictions are enforced, are likely the first one to be banned. Other recommended risk reduction measures are good agricultural practices including the method of irrigation (e.g. drip irrigation) intended to limit water contact on consumable parts of produce. Keraita *et al*. [166] have also proposed the use of perforated caps, or filters at the spouts of watering cans to reduce splashes of soil onto produce, though this intervention still requires to be tested on a larger scale to assess its practical feasibility and effectiveness in reducing produce contamination. Farmers did not practice irrigation cessation as the value of the salad crops depends on how fresh the vegetables look prior to harvest [7]; besides the practice has been found to reduce crop yield and farmer income [153].

Poor sanitation and hygiene is often associated with produce contamination though in this study no association between environmental exposures, such as distance to open drains and refuse, and produce contamination at markets was found. This finding was similar to an earlier field trial in Accra where Amoah *et al*. [48] found that the poor sanitation conditions and practices at markets did not significantly increase the level of contamination of farm produce sold at markets. This study did not find evidence to support hygienic practice, however vendors should be encouraged to wash produce under running water, or use multiple batches of water to wash produce. Clean water should be made more readily available at markets, and where possible disinfectants that are readily available and relatively cheap could also be used to further reduce produce contamination. However, disinfectants could possibly change the taste, and this should be further explored and tested, before being promoted. Hygiene certification should be encouraged, as it showed to have an impact on produce quality, with those owning a certificate having shown to have cleaner food; possibly because they have to adhere to food hygiene and safety rules, or else their certificate will be withdrawn.

Another opportunity that was identified was the creation, or strengthening of communication channels/platforms to overcome mistrust between farmers, salespersons, consumers, municipality officers, the media and the general public. This would, however, only work if farmers' livelihoods are recognized.

6.5 Conclusions

Based on the WHO guidelines, this study showed that there was potential health risk to wastewater farmers and consumers of wastewater irrigated produce, and hence the need to intervene appropriately to reduce these risks. The results of the study were, however, inconclusive to provide sufficient evidence on the actual risks to farmers and consumers, and hence additional behavioural and microbial exposure data are required to further refine and improve the robustness of risk estimates from QMRA models. Access to credit schemes and improved land security are recommended measures to encourage farmers to adopt risk reduction measures. For consumers, risk reduction interventions should be prioritised at markets and kitchens, due to the high levels of contamination on produce/salad at these sites, and the comparatively easier implementation of risk reduction measures at these domains compared to the farm level. Recommended measures should include hygiene inspection, hygiene certification and general environmental and food hygiene practices.

6.6 Novelty of study and contributions to knowledge

There were several methodological strengths of this study which have helped to provide information useful in the field of wastewater irrigation, health risk assessment and food hygiene and safety. Chapter two of this thesis presented a first time faecal exposure assessment of wastewater farmers using direct observations. Further, the information gathered from the direct observations were triangulated or validated through questionnaires and focus group discussions. In as much as the observations were conducted only once and for only 3 hours per farmer and hence might not reflect the actual exposure patterns, the results have nonetheless provided vital information on high risk farming activities, and the frequencies that farmers are actually in direct contact with faecal pathogens through wastewater or wastewater contaminated soil. More importantly, the exposure assessment have also provided information on farmers direct hand to mouth events to contaminated soil which is a key premise of the WHO guidelines for assessing farmers occupational health risk through wastewater farming.

In Chapter 3, the study also conducted a first time farm-to-fork assessment of produce hygiene and quality status in four different disease transmission domains (wastewater irrigated fields, markets, street food vending sites and restaurants/hotels). Of particular interest in the approach is that all data were collected in the same study area within the same time period and season. This approach provides a better and a more reliable way of comparing produce contamination levels and health risk estimates across different disease transmission domains. This was identified as a limitation in previous studies which often included and compared produce quality levels only between the farm and market levels, or compared with other pathways such as street kitchens but using different studies from different study areas and also conducted at different time periods and seasons [48, 65]. The current study was however limited in its design as produce sampled at markets, street kitchens and restaurants were not necessarily from the same lot or batch as those sampled from farms. It therefore remains unclear on the relative impact of wastewater irrigation and post-harvest contamination of produce.

In the risk perception Chapter (Chapter 4), the study assessed participants' awareness and knowledge levels of health risks, the sources of produce and irrigation water and what motivates buyers and consumers to buy produce or salad from vendors. Unlike previous studies [71, 141, 182], this study further assessed how awareness of these sources and other health risk indicators influenced participants to adopt health protective measures or influence their decision to buy or consume wastewater irrigated produce. This data is considered vital as it could guide policy makers and other health promoters on how best to design their risk reduction interventions programs. More importantly, these findings could serve as a guide on the promotion of the WHO multiple barrier approach as a health risk intervention approach.

In the last results Chapter (Chapter 5), the study performed a quantitative microbial risk assessment of consumers of wastewater irrigated produce and for the first time used direct concentrations of pathogens (norovirus) in wastewater, contrary to most previous studies [66, 195, 196, 200] which have adopted the indicator-pathogen ratio approach or other approaches such as the human faecal shedding rate. Additionally, the pathogen infection risk was modelled as a probability of exposure to both positive and negative samples, and not only positives which could potentially overestimate the risk. Further, most of the input data including the quantity and frequency of exposure originated from the same dataset from the same study area which potentially reduces the level of uncertainty in the risk estimates compared to the predominant use of data from different datasets from different geographical areas. Finally, the study performed a risk comparative assessment between the use of direct pathogens in wastewater and the use of indicator-pathogen QMRA water models in order to determine their health risk impact on consumers.

6.7 Further research

The following areas of research are proposed based on the study findings and limitations:

1. Estimation of farmers' occupational risk by incorporating farmers' direct contact to faecal contamination and also including incidences of hand to mouth contact in QMRA models. For example, the quantity of soil ingested by wastewater farmers could be estimated by modelling it as the product of the number of hand-to-mouth events and the estimated soil transfer rate per hand contact [201]. This is then combined with all the other input parameters to estimate the pathogen risk of infection among wastewater farmers.
2. A detailed assessment on the effect of produce washing practices on produce contamination at markets and also the effect of salad preparation and management practices on its contamination at street kitchens and restaurants. This assessment can be conducted either as a field trial or as a case control study where cases will be vendors who wash their produce at markets, or those who would be guided on the hygienic method of preparing and cleaning salad in the case of street food vendors.
3. Field trials or related study designs to assess the impact of wastewater irrigation on produce quality beyond farm level (markets and kitchens). In this case, it would be more appropriate to identify study settings or areas where significant number of cases (those practicing wastewater irrigation) and controls (farmers using non-wastewater sources) could be identified. Two groups of vendors from markets and also from street kitchens would then be selected to purchase produce from the two groups of farmers after which the relative influence of wastewater, market handling and hygiene practices at kitchens would be assessed.
4. Prospective cohort studies to determine the association of wastewater use and the incidence of diarrhoea diseases or helminth infections among farmers.
5. Assessment of the impact of government and other stakeholders' collaboration and support with farmers and vendors on the adoption of risk reduction measures at farms, markets and food establishments. This assessment can also be conducted as part of a case control study where cases would receive the required support and collaborations

from government and other key stakeholders and controls would receive no such support.

6. Investigations to determine the presence and concentrations of viruses, helminths and other pathogens in irrigation water, farm soil, raw produce and prepared salad. Where resources are available, it is vital that these subsequent studies focus more on determining the actual prevalence and concentrations of pathogens in these environmental samples especially produce at markets and ready to eat salads at kitchens.
7. Behaviour change studies including the use of emotional drivers such as disgust to assess the uptake of health protective measures by farmers, salespersons and consumers of irrigated produce. The approach and lessons learnt on the use of disgust and other emotional drivers in the promotion of hand washing with soap and the adoption of the community total led sanitation can be assessed and be applied to wastewater farmers and salespersons of produce and ready to eat salad to influence their adoption of health protective measures.

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Annex 1: Environmental and food sample field collection forms

Annex 1a: Irrigation water sample collection form

Irrigation Water Sample Collection Form

Barcode/sample Id [ ]

Date [ ] / [ ] / [ ]

Time [ ] [ ]

1. GPS latitude [ ]
2. GPS longitude [ ]
3. Location ID: [ ]

Location Description [ ]

4. What is the source of irrigation water collected?

Drain Water ☐ Shallow Well ☐
Channelled Stream/River ☐ Tube/Deep Well ☐
Dug-out/Pond ☐
Piped Water ☐

5. If "Yes", check box:

Within 3m of trash? ☐
Within 3m of faeces? ☐
Within 30m of latrine or Defaecation area? ☐

Field Notes: [ ]

6. Physiochemical Characteristics

Turbidity [ ]

Temperature [ ]

Electrical conductivity [ ]

Salinity and pH [ ]

Lab notes: [ ]

7. At lab:

Starting Volume (L) [ ]

Personal Information

8. Sex Male ( ) Female ( )
9. Age (yrs) ..[ ]
10. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
11. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Date: **Time:**

Enumerator ID: **Location ID:**

Annex 1b: Particulate (soil) sample collection form

Particulate (Soil) Sample Collection Form

Barcode/sample Id

Date / /

Time

1. GPS latitude
2. GPS longitude
3. Location ID:

Location Description

4. If "Yes", check box:

Exposed to sunlight? ☐
Within 3m of faeces? ☐
Ask farmer if soil is contaminated with poultry manure ☐

Field Notes:

5. At lab:

Weight (g)

Personal Information

6. Sex Male ( ) Female ( )
7. Age (yrs) ...
8. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
9. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Date: **Time:**
Enumerator ID: **Location ID:**

Annex 1c: Raw produce (farm) sample collection form

Raw Produce (Farm) – Sample Collection Form

Barcode/sample Id ☐

Date ☐ / ☐ / ☐

Time ☐ ☐

1. GPS latitude ☐
2. GPS longitude ☐
3. Location ID: ☐
4. Select the source of raw produce
   Farm ☐
   Market ☐
   Home ☐
5. Tick the type of raw produce
   Lettuce ☐ Carrot ☐
   Cabbage ☐ Spring Onion ☐
6. Enter the number collected: ☐
7. Indicate when (in hours) irrigation was last done on produce before sampling: ☐
8. How produce placed in whirl-pak bag (select one):
   Plastic or paper wrap ☐
   Hands ☐
   Other ☐ Specify: ☐
9. Mark if the sample was taken
   Within 3m of faeces ☐
   Within 30m of latrine ☐
   With flies on food ☐
   Within 3m of sewage outfall Or open drain ☐
10. At lab:
   Weight (g) ☐ or Volume (mL): ☐

11. Mark how produce was covered
   Covered ☐
   Not covered ☐

12. Produced exposed to Sunlight:
   Yes ( ) No ( )

Notes: ☐

Personal Information

13. Sex Male ( ) Female ( )
14. Age (yrs) .. ☐
15. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
16. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Date: **Time:**

Enumerator ID: **Location ID:**

Annex 1d: Raw produce (market) sample collection form

Raw Produce (Market) - Sample Collection Form

Barcode/sample Id ______ Date ___/___/___

Time ___ ___

1. GPS latitude ______
2. GPS longitude ______
3. Location ID: ______
4. Select the source of raw produce
   Farm ☐
   Market ☐
   Home ☐
5. Tick the type of raw produce
   Lettuce ☐
   Cabbage ☐
6. Enter the number collected: ☐
7. Type of market vendor : a) At main market (under shed) b) Open/street market (outside main market)
8. A). Indicate how long (hours) produce has been stored ______
   B). Record produce storage temperature ______
9. How produce placed in whirl-pak bag (select one):
   Plastic or paper wrap ☐
   Hands ☐
   Other ☐ Specify: ______

10. Mark if the sample was taken
Within 3m of faeces ☐
Within 30m of latrine ☐
With flies on food ☐
Within 3m of sewage outfall Or open drain ☐

11. At lab:
Weight (g) ______ or Volume (mL): ______

11. Mark how produce was covered
Covered ☐
Not covered ☐

12. Produced exposed to Sunlight:
Yes ( ) No ( )

13. How produce is displayed: a) On the ground b) >1m above ground c) <1m above ground d) Other

14. Is vending site concreted?
Yes ( ) No ( )

Notes: ______

Personal Information

16. Sex Male ( ) Female ( )
17. Age (yrs) .. ______
18. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
19. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Annex 1d: Food (salad) sample collection form

Food (Salad) Sample Collection Form

Barcode/sample Id ☐ Date ☐/☐/☐

Enumerator ID: ☐ Time ☐ ☐

1. GPS latitude ☐
2. GPS longitude ☐
3. Location ID: ☐
4. Select the source of ready-to-eat food
   Street food ☐
   Hotel ☐
   Restaurant ☐
5. Select type of vendor
   Mobile ☐
   Non-mobile ☐
6. Indicate how long (days/hrs) produced has been stored before using for salad ☐
7. Source of vegetables used to prepare salad: a) Farm gate b) Wholesale market c) Retail market d) Other (Specify): ☐
8. How food was placed in whirl-pak bag (select one):
   Plastic or paper wrap ☐
   Spoon/spatula ☐
   Hands ☐
   Other ☐ Specify: ☐
9. How salad was treated: a) Salt water b) Vinegar c) Salt Water & vinegar d) water e) other (specify) ☐
10. Mark if the sample was taken
    Within 3m of faeces ☐
    Within 30m of latrine ☐
    With flies on food ☐
    Within 3m of sewage Outfall Or open drain ☐
    Within 3m of refuse ☐
11. At lab:
    Weight (g) ☐ or Volume (mL): ☐

12. Mark how salad was covered
    Covered ☐
    Not covered ☐
13. Is vending site concreted? Yes ( ) No ( )
14. If hotel, class of hotel ☐
15. Do you have hygiene permit to operate? Yes ( ) No ( )
16. If yes, from where? a) FDA b) AMA c) Other (specify)..

Notes ☐

Personal Information

17. Sex Male ( ) Female ( )
18. Age (yrs) ..☐
19. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
20. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Annex 2: Questionnaires for wastewater farmers, market and street food vendors, and consumers of salad produce

Annex 2a: Questionnaire for farmers

QUESTIONNAIRE FOR FARMERS.

A. Farming Practices

1. How many days do you normally work on the farm in a week? [ ]

2. On average, how many days do you work on the farm in a month? [ ]

3. Can you estimate how many months you work in a year? [ ]

4. How long do you normally spend doing the following farm activities on a daily basis?

Farm Activity	4i. Average time spent (in minutes)	4ii. Number of beds (Average)
a) Irrigation/Watering		
b) Soil tilling/Forking		
c) Applying poultry/cow-dung manure		
d) Transplanting		
e) Bed Preparation		
f) Removing Weeds		

5. How often do you perform the following activities in a week or month?

Farm Activity	Indicate frequency of activity (days per month)
a) Irrigation/Watering	
b) Soil tilling/Forking	
c) Applying poultry/cow-dung manure	
d) Bed Preparation	
e) Removing Weeds	

6. How often do you plant (transplant) the following vegetables in a year?

   a). Lettuce [ ] b). Spring Onion [ ] c). Cabbage [ ]

B. Environmental Hygiene conditions

7. Where do you normally defaecate when at work on the farm?
   Public toilet ( ) toilet on the farm premises ( ) A neighbour's toilet ( )
   Open Defaecation ( )
8. What is your source of drinking water when working on the farm? (Tick all responses)
   Sachet water ( ) Bottled mineral water ( ) Piped water ( ) Water from home ( )

C. Food Hygiene practices

9. How many times do you eat when at work? Once ( ) 2 times ( ) 3 times ( )
   More than 3 times ( )

10. What is the source of the food you normally eat when at work?
    Food from home ( ) street food ( ) local restaurant food ( ) prepared food on farm ( ) Other ( )
11. Which of these do you do most?
    Eating at the farm ( ) Eating at the food vending site ( ) Eating at home ( ) Other ( )
12. Do you normally wash your hands before eating at the farm site?
    Yes ( ) No ( )
13. If Yes, what do you wash your hands with?
    Irrigation Water only (Specify)....................... Pipe water only ( ) Sachet Water only ( ) Soap and water ( )

D. Health Risk Perceptions and Awareness

14. Are you aware of any health risks associated with farming practices using drain water? Yes ( ) No ( )
15. If yes, what kind of health risk do you know of?
    List all of them...
16. What do you do to protect yourself from these health risks?
    List them..

E. Health status

17. What is the most common disease you experience every year?
    Diarrhoea ( ) Cholera ( ) Malaria ( ) Skin disease ( ) Worm infections ( ) Other (Specify)
18. Have you had diarrhoea within the last 2 weeks?
    Yes ( ) No ( )
19. Have you had any skin diseases within the last 6 months?
    Yes ( ) No ( )
20. Have you had any worm infections within the last 6 months?
    Yes ( ) No ( )

F. Socio-economic issues

21. Does the use of drain water/dug-out water for irrigation increase your income as compared to the use of piped water?
    Yes ( ) No ( ) Cannot tell ( )
22. Is farming the main source of your income? Yes ( ) No ( )
23. How much time do you spend on the farm?...........
24. If you have any other job, how much time do you spend at :
    Farm...................... Other job (s)............................
25. How many people depend on you? [ ]

G. Personal Information

26. Sex Male ( ) Female ( )
27. Age [ ]
28. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) Other ( )
29. Educational background: Illiterate/no formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )

Date: **Time:**

Enumerator ID: **Location ID:**

Annex 2b: Questionnaire for market vendors

QUESTIONNAIRE FOR MARKET VENDORS

A. Selling information

1. How many days in a week do you normally sell vegetables at the market? [ ]

2. Where do you normally buy your vegetables from? (Accra, Kumasi, Togo etc)

Vegetables	Source (Where)
a) Lettuce	
b) Carrots	
c) Spring Onions	
d) Cabbage	

3. On average, how many of these vegetables do you sell in a day?

Vegetables	i. Number sold in a day	ii. No. of sacks
a) Lettuce		
b) Carrots		
c) Spring Onions		
d) Cabbage		

4. Of the four vegetables above, which of them is bought most by customers?
   Lettuce ( ) Carrot ( ) Spring onion ( ) cabbage ( )

B. Produce Hygiene conditions

5. Where do you normally store your produce before selling them?
   At the market ( ) At home ( ) other ( ) specify
6. How do you normally store the following vegetables before selling them? (Indicate in the table below):

Vegetables	How storage is done
a) Lettuce	
b) Carrots	
c) Spring Onions	
d) Cabbage	

In a sack ( ) In a basket ( ) On a table but covered ( )
In a box ( ) Other ( ) specify...

7. Where do you normally display your vegetables for sale? (Indicate in the table below):

Vegetables	How produce is displayed
a) Lettuce	
b) Carrots	
c) Spring Onions	
d) Cabbage	

Table top ( ) Basket/bowl ( ) material on ground ( ) bare ground ( )

8. Do you wash the vegetables before sales?
   Yes ( ) No ( )
9. If yes, what is the source of water for washing the vegetables?
   Piped water ( ) well water ( ) Tanker services ( ) Other (specify)...................
10. How much time do you spend on the following activities daily

Activity	Time Spent
a) Washing of lettuce	
b) Washing of carrot	
c) Cutting or removal of waste parts of cabbages	
d) Removal of waste parts of spring onions	

11. Are customers normally happy with the quality of vegetables sold at the market?
    Yes ( ) No ( )
12. What do customers normally complain of when at the market?
    Write complaints...
    ....

C. Environmental Hygiene conditions

13. Where do you normally defaecate when you are at the market?
    Public toilet ( ) Market toilet ( ) In a polythene bag ( ) Open Defaecation ( )
14. What is your source of drinking water when working at the market?
    Sachet water ( ) Bottled mineral water ( ) Piped water ( ) Water from home ( )
15. Are you normally satisfied with refuse collection and management at the market?
    Yes ( ) No ( )
16. If No, what are you not satisfied with?
    Write down reason (s)..
17. Are you generally satisfied with drainage management at the market?
    Yes ( ) No ( )
18. If No, what are you not satisfied with?
    Write down reason (s)..

D. Hand washing and food Hygiene practices

19. How many times do you eat when at the market?
    Once ( ) 2 times ( ) 3 times ( ) more than 3 times ( )
20. What is the source of the food you normally eat when at the Market?
    Food from home ( ) street food ( ) local restaurant food ( ) Other ( ) specify.......................................
21. Do you normally wash your hands when eating at the market?
    Yes ( ) No ( )
22. If Yes, what do you wash your hands with?
    Only water ( ) Soap and water ( )

E. Health Risk Awareness and Perceptions

23. Where do you buy the vegetables you sell from?
    Farm gate ( ) Wholesale market ( ) Retail Market ( ) Other ( )
24. If farm gate, do you have any reason (s) why you buy from these farm gates?
    Yes ( ) No ( )
25. If yes, what are your reasons?
    List reasons...

26. Do you know of the source of water farmers use to irrigate their crops?
    Yes ( )   No ( )
27. If you know the source is drain water, would you still buy the vegetables?
    Yes ( )   No ( )
    List reasons for Yes or No answer..
28. Are you aware of any health risks associated with the consumption of vegetables that are irrigated with drain water?   Yes ( )   No ( )
29. If Yes, what health risks do you know of?
    List health risks...
30. Do you consume some of the vegetables yourself?   Yes ( )   No ( )
31. If you use the vegetables yourself, how many times in a week do you consume them uncooked (i.e. prepare it as salad)? [ ]

32. How much of the following vegetables do you use to prepare salad for one meal?

Vegetable	**Quantity used**
a) Lettuce	
b) Cabbage	
c) Spring Onion	
d) Carrot	

F. Personal Background

33. Sex: Male ( )   Female ( )
34. Age (yrs): [ ]
35. Religion: Christian ( ) Moslem ( ) Traditional worshipper ( ) others ( )
36. Education: Illiterate ( ) Primary education ( )   JHS/MS ( )
    SHS/A-level ( ) Polytechnic/University ( )

Date: **Time:**

Enumerator ID: **Location ID:**

Annex 2c: Questionnaire for produce buyers (market)

QUESTIONNAIRE FOR PRODUCE BUYERS (MARKET)

A. Consumption Patterns

1. Which of these vegetables do you normally buy most at the market? Lettuce ( )
   Carrot ( ) Spring onion ( ) cabbage ( )
2. How often in a week do you buy lettuce/cabbage from the market? [ ]
3. What do you normally do at home to clean these vegetables?
   Wash with clean water ( ) Wash in salt water ( ) Add a disinfectant ( )
   Other ( ) Specify...
4. Mostly, do you consume these vegetables cooked or uncooked?
   Cooked ( ) Uncooked ( )
5. If vegetables are sometimes consumed uncooked, how often in a week is this done (i.e. consumed as salad)? [ ]

6. On average, how many of these vegetables do you use for one salad meal in a day?
   a). Lettuce [ ] b). Cabbage [ ] c). Spring Onion [ ] d). Carrot [ ]

7. How many people in the house consume the salad in a day? [ ]

B. Health Risk Awareness and Perceptions

8. Are you aware of the source of the vegetables you buy at the market? Yes ( ) No ( )
9. What is the main reason why you buy from this market vendor and not from others?
   Write down the main reason..
10. Do you think the source of water farmers use to irrigate the vegetables could have influence on your decision to buy them? Yes ( ) No ( )
11. Would you still buy these produce or not if you were aware that it was irrigated with wastewater (drain water)? Buy ( ) Not buy ( )
12. Are you aware of any health risks associated with the consumption of produce that are irrigated with wastewater? Yes ( ) No ( )
13. If Yes, what are the main health risks/disease you know of?
    Write down main health risks mentioned...
    ....
14. Have you had diarrhoea within 2 weeks after consuming salads before?
    Yes ( ) No ( ) cannot remember ( )

C. Environmental Conditions and Health Status

15. Are you generally satisfied with how produce are displayed for sale at the markets?
    Yes ( ) No ( )
16. Are you satisfied with the general environmental conditions at the produce vending site?
    Yes ( ) No ( )
17. If No, can you give reasons?...
    ....
18. What do you think can be done by market vendors to improve environmental sanitation at the market?
    Write down responses...
    ....

19. What do you think can be done by the government to improve environmental sanitation conditions at the market?
    Write down responses..

D. Personal Background

20. Sex: Male ( ) Female ( )
21. Age (yrs): $\boxed{}$
22. Religion: Christian ( ) Moslem ( ) Traditional worshipper ( ) others ( )
23. Occupation: Government/Office worker ( ) Trading ( ) Vocational ( ) Other ( )

Enumerator ID: **Location ID:** **Date:**

Annex 2d: Questionnaire for street food vendors

QUESTIONNAIRE FOR STREET FOOD VENDORS

A. Selling information

1. How many days in a week do you normally sell cooked rice/other food with salad? [ ]

2. On average, how much of these vegetables do you use to prepare salad for a day?
   a). Lettuce [ ] b). Cabbage [ ] c). Carrot [ ] d). Spring Onion [ ]

3. On average, how many customers buy rice with salad in a day? [ ]
4. How much time do you spend selling food at your vending site? [ ]

5. What do you normally do when less busy at your vending site?
   Chatting with friends ( ) Washing of utensils ( ) wait for customers ( )
   Other ( ) Specify...

B. Produce and Food Hygiene Practices

6. Where do you normally store the produce before using them for salad?
   At home ( ) At vending site ( ) use it immediately after buying ( )
   Other (Specify)
7. How do you store the produce? On the bare ground ( ) On a mat on the ground ( )
   In a box or container ( ) Other (Specify)
8. Where do you normally prepare the salad?
   At home ( ) At vending site ( ) Other ( ) Specify...
9. What do you normally do to clean the vegetables before salad preparation?
   Wash with clean water ( ) Wash in salt water ( ) Use a disinfectant (Vinegar).................. Other (Specify)................................
10. What is the source of water for washing the produce before salad preparation?
    Piped water ( ) well water ( ) Tanker services ( ) Other ( ).
11. Do you normally wash your hands before salad preparation? Yes ( ) No ( )
12. If Yes, what do you use to wash your hands? Only water ( ) Water and Soap ( )
    Other ( )
13. What is your normal practice of serving the salad to consumers?
    Bare hands ( ) Hand covered with a polythene bag ( ) Spoon/ladle ( ) Other ( )

C. Environmental Hygiene conditions

14. Where do you normally defaecate when you are at work on the farm?
    Public toilet ( ) Neighbour's/friend's toilet ( ) In a polythene bag ( ) Open Defaecation ( )

D. Health Risk Awareness

15. Where do you normally buy the vegetables you use to prepare the salad from?
    Farm gate ( ) Wholesale market ( ) Retail Market ( )
16. If farm gate, do you have any reason (s) why you buy from these farm gates?
    Yes ( ) No ( )
17. If yes, what are your reasons?
    List reasons...
18. Are you aware of the source of water farmers use to irrigate their crops?

Yes ( ) No ( )

19. If you know the source is drain water, would you still buy these vegetables?
Yes ( ) No ( )
20. If yes, could you provide some reasons...
....
21. If No, could you provide some reasons...
....
22. Are you aware of any health risks associated with the consumption of vegetable salads that are irrigated with wastewater? Yes ( ) No ( )
23. If Yes, what health risks do you know of?
List health risks...
24. Do you consume some of the salads yourself? Yes ( ) No ( )
25. If yes, how often in a week do you consume them? ☐

26. Have you had any diarrhoea disease within 2 weeks after consuming salad foods?
Yes ( ) No ( ) cannot remember ( )

E. Personal Background

27. Sex Male ( ) Female ( )
28. Age (yrs) ☐
29. Religion Christian ( ) Moslem ( ) Traditional worshipper ( ) others ( )
30. Education Illiterate ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( ) Polytechnic/University ( )
31. Do you do any other work apart from street food vending? Yes ( ) No ( )
32. If Yes, how much time do you spend on each of them?
Street food vending.......................... Other jobs..

Enumerator ID **Location ID** **Date**

Annex 2e: Questionnaire for street food consumers

QUESTIONNAIRE FOR STREET FOOD CONSUMERS

A. Consumption Patterns

1. Do you normally take the salad or only the rice? Yes, I take ( ) No, I do not take ( )
2. If No, do you have any reasons why you don't take the salad?
   Write down reason(s)...
   ....
3. If you take the salad, how often in a week do you normally consume street salad food ("check-check"/others)? [ ]

4. What makes you prefer to buy and consume street foods with salad? (tick all answers)
   Cheap ( ) Convenient ( ) I just like it ( ) Other (specify).................

B. Health Risk Awareness and Perceptions

5. Does anything influence your decision to buy the food from one seller and not the other?
   Write down the main reason...
   ....
6. Are you aware of the source of water used to irrigate the vegetables used for the salad?
   Yes ( ) No ( )
7. Would you still buy or take the salad if you were aware that the vegetables used to prepare it were irrigated with drain water? Buy ( ) Not buy ( )
8. Are you aware of any health risks associated with the consumption of salad prepared from vegetables that are irrigated with drain water? Yes ( ) No ( )
9. If Yes, what is the main health risk/disease you know of? Write down the main health risk/disease mentioned
   ....
   ....

C. Environmental Conditions and Health Status

10. Are you generally satisfied with how the salads are prepared?
    Yes ( ) No ( ) don't know how they are prepared ( ) Other ( )
11. Are you satisfied with the general environmental conditions at the food vending site?
    Yes ( ) No ( )
12. If No, what do you think can be done to improve the environmental sanitation conditions at the vending sites?...
    ....
13. Have you ever had diarrhoea disease within 2 weeks after consuming lettuce salads?
    Yes ( ) No ( ) cannot remember ( )

D. Personal Background

14. Sex: Male ( ) Female ( )
15. Age (yrs): [ ]
16. Religion: Christian ( ) Moslem ( ) Traditional worshipper ( ) others ( )
17. Occupation: Government/Office worker ( ) Trading ( ) Vocational ( )
    Other (specify)

Enumerator ID **Location ID** **Date**

Annex 3: Focus group discussion guides for farmers and market vendors

Annex 3a: Focus group discussion with farmers

FOCUS GROUP DISCUSSION WITH FARMERS

A. Farming Practices

I will start the discussions by asking you to tell me more about your daily farming activities. Let's think of it from the time you arrive on the farm in the morning until you finish all our activities and leave for home in the afternoon or evening *(Probe for the following):*

1. What are the key activities farmers perform on the farm *(let farmers mention about four activities they consider to take most part of their working time)*
2. When do farmers do most of their farming activities (morning, afternoon or evenings?)
3. How much time do farmers spend on the four key activities they mentioned above?
4. How often (in a week) do farmers perform each of the four key activities you have mentioned?
5. What is the single activity farmers consider to take the most part of their time?
6. On average, days farmers work in a week and months in a year
7. How much time farmers spend on the farm daily?
8. What do farmers do when less busy on the farm?

B. Environmental Hygiene conditions

Now I would want us to discuss about your water and sanitation practices on the farm. How would you describe your water and sanitation situation when at work on the farm? (Discuss the following):

9. Availability of toilet facilities on the farm and where farmers defaecate *(defaecation practice – public toilet, open defaecation etc., are farmers satisfied with where they defaecate, what are they not satisfied with?, what do they think can be done to improve the current situation, are they concerned about where they defaecate or not)*
10. Availability of drinking water *(what is the source of drinking water, where do farmers get drinking water from, are they concerned about what they drink – quality issues)*
11. Are farmers normally satisfied with the general environmental conditions (presence of visible faeces etc.) at the farm?

C. Hand washing and Food Hygiene practices

Now that I know much about your farming practices and environmental hygiene, can we now discuss how you wash your hands and also eat at the farm? Is hand washing and food hygiene at the farm a concern to farmers? *(Discuss the following):*

12. What food do farmers normally eat, where do they get these food from, where do they eat it, when do they eat at the farm,
13. Food hygiene (are food normally eaten hot or cold, are food covered, are the vending sites clean?)
14. Do farmers wash their hands at the farm? *(what are the critical times/activities that make farmers wash their hands, what do they use to wash their hands, why do they wash their hands or not)*

D. Health Risk Perceptions and Awareness

Can we now discuss about what you think can be a health risk as you work as farmers?

15. What do you think about your farming practices and health risks? (Are farmers aware of any health risks to their practices, what activities do they consider as critical and can pose health risk to them, what health risks or disease are they aware of?
16. What kind of exposures are farmers aware of (soil, wastewater, produce, chemicals etc.)?
17. Do farmers consider exposure to faecal contamination as health risks? *(find out what they think – why, how are they exposed to faecal contamination, what they think exposes them more to faecal contamination at the farm level, how long do they think they are exposed to faecal contamination?)*
18. What can you do to protect yourselves from these health risks *(discuss irrigation methods and practices, the use of protecting clothing etc.)*
19. What do farmers think about consumption of drain water irrigated produce and health risks?
20. What do farmers consider the municipalities or government to do to support their farming activities?

E. Health status

21. Discuss disease prevalence among farmers *(what are the common diseases you experience as farmers, if not mentioned already - have farmers experienced diarrhea diseases, worm infections and skin diseases before, do they attribute it to their farming practices?)*
22. What about members of your family - Has any member of your household experienced any health problems that can be related to the wastewater irrigation practices?

F. Socio-economic issues

Now we are at the final part of our discussion and I would want to know of the benefits you get in your work.

23. How do you relate the use of wastewater (drain water) and your income levels? *(Do farmers think the use of wastewater increases their income or not, what makes them think so or otherwise?, is farming their main source of income, are they the bread-winners of their family).*
24. "Why do you use this source of water (drain water or dug-out) for irrigation instead of piped water or other water sources?

Now we have come to the end of our discussion. Thank you all for your time and contributions.

Enumerator ID **Location ID** **Date**

Annex 3b: Focus group discussion with market vendors

FOCUS GROUP DISCUSSION WITH MARKET VENDORS

A. General Market Practices

I will start our discussion by asking you to tell me more about your daily market activities. We do this by considering all what you do from the time you start work until you leave for your various homes (probe for the following):

1. Working hours *(when they start work and close, how much time they normally spend at the market)*
2. Type of produce sold *(what produce do you normally sell, why do you sell these produce (availability, good market etc.), how many produce sold in a day, how often produce are sold in a year)*
3. What are your typical market activities related to salad vegetables *(what you do in the morning, afternoon, evening, what you do when less busy?)*
4. Market activities and time (*Which three or four of the market activities related to salad vegetables take more time?)*
5. Working days and months *(On average, how many days in a week and months in a year do you work at the market)*

B. Produce Hygiene conditions

Now that I know more about what you normally do for your working period at the market, I would want us to talk about how you care for your produce at the market. Can you describe how you ensure that produce are kept and sold clean to customers? (Discuss the following):

6. Where produce are stored, where they are displayed *(find out for lettuce, cabbage, spring onion and carrots)*
7. Do you wash or clean them and if so how? (for each of the above vegetables)
8. Customer satisfaction *(are customers generally satisfied with quality of produce, what do they normally complain of, why do they complain of those)?*

C. Environmental Hygiene conditions

Now we can discuss about the environmental sanitation conditions at the market.

9. Can you describe the sanitation situation at the market? *(availability of toilets, where defaecation is done, defaecation practices, cleanliness of defaecation places, distance and time spent to attend to toilet, general satisfaction about toilet facilities)*
10. What about our drinking water supply at the market *(availability of drinking water, type of water supply, source of drinking water, quality of drinking water, distance or time spent to get drinking water etc)*
11. How will you describe refuse collection and management at the market? *(availability of skip containers, who does the collection, is collection done frequently, does it pose a health risk)*
12. Is drainage management at the market of concern? *(Availability, who cleans them, hygienic conditions/breeding grounds for flies and vectors?)*

13. What do you think can be done to improve environmental sanitation systems and management at the market? *(What must be done, who should do what, what should the roles and responsibilities of vendors?)*

D. Hand washing and food Hygiene practices

Can you also tell me more about your hand washing and food hygiene practices at the market?

14. What food do market vendors normally eat when at the market, where do they get these food from, where do they eat it, when do they eat?)
15. Food hygiene (are food normally eaten hot or cold, are food covered, are the vending sites clean?)
16. Do market vendors wash their hands at the market? *(What are the critical times/activities that make market vendors wash their hands at the market, what do they use to wash their hands, why do they wash their hands or not?).*

E. Health Risk Awareness and Perceptions

We are at the final part of our discussion and I would want to learn more about what you think can constitute health risks as you work at the markets.

17. Do you think the market environment poses health risks to the market vendors and people who come to the market? *(Probe on the following: why they pose as risks, how are people exposed to these risks factors, what do they constitute as the hazards or risk factors?)*
18. What can you say about irrigation water for vegetables and health risks *(are vendors aware of the source of irrigation water for vegetables, does the source of irrigation water influence their decision to buy produce from farm-gates or wholesale markets, do they think there are any disease/health risks, what type of risks (direct or indirect), who is at risk?)*
19. Tell me about disease prevalence among vendors *(what are the common diseases that you experience as market vendors, if not mentioned already – get the prevalence of diarrhea and worm infections among vendors, whether vendors associate these diseases to general environmental conditions at the market or exposure to wastewater irrigated produce?)*
20. Health protection measures *(what vendors think can be done to protect themselves and other people at the market from health risks, what do they expect from the municipality or government?)*

Now we have come to the end of our discussion. Thank you all for your time and contributions.

Enumerator ID **Location ID** **Date**

Annex 4: Behaviour observation guides for farmers, market and street food vendors

Annex 4a: Observation guide for farm workers

Observation Guide/Behaviour Record for Farm Workers
For each activity, record the time for performing the activity and also complete the exposure parameters. Observe the farmer from 7 – 10am.

Activity	**Time period for activity**	**Exposures**	**Time period for Exposures**
1. Bed Preparation	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( ) c) Hand contact with face/mouth: Yes ( ) No ( )	Hand contact time: Feet contact time: Tally for hand contact:
2. Transplanting	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( ) c) Hand contact with face/mouth: Yes ( ) No ( )	Hand contact time: Feet contact time: Tally for hand contact
3. Soil tilling (Forking)	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( ) c) Hand contact with face/mouth: Yes ( ) No ( )	Hand contact time: Feet contact time: Tally for hand contact:
4. Removal of weeds	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( ) c) Hand contact with face/mouth: Yes ( ) No ( )	Hand contact time: Feet contact time: Tally for hand contact:
5. Watering (Irrigation) of crops (indicate the source of irrigation water, e.g. drain water)	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( ) c) Hand in contact with irrigation water: Y/N d) Feet in contact with irrigation water: Y/N	Hand contact time: Feet contact time: Hand contact time: Hand contact time:

6. Mixing of chemicals (pesticides/insecticides) for spraying	Start time: End time:	a) Farmer in hand gloves: Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( )	
7. Spraying	Start time: End time:	a) Farmer in nose mask : Yes ( ) No ( ) b) Farmer in hand gloves: Yes ( ) No ( ) c) Feet in contact with soil: Yes ( ) No ( )	Feet contact time:
8. Harvesting (including packing of produce into sacks)	Start time: End time:	a) Hand in contact with soil : Yes ( ) No ( ) b) Feet in contact with soil: Yes ( ) No ( )	Hand contact time: Feet contact time:
9. Transportation of harvested produce to road side	Start time: End time:	a) Feet in contact with soil: Yes ( ) No ( )	Feet contact time:
10. Eating	Start time: End time:	a) Farmer washed hands: Yes ( ) No ( ) b) Farmer washed hands with water and soap: Yes ( ) No ( ) c) Farmer washed hands with only water: Yes ( ) No ( )	
11. Farmer Idle (Indicate activities)	Start time: End time:		

Enumerator ID: ***Location ID:*** ***Date:***

Annex 4b: Observation guide for market vendors

Observation Guide – Behaviour Record (Market Vendors)
For each activity, record the time for performing the activity and also complete the exposure parameters. Observe the Vendor from 7 – 10am.

Activity	**Time period for activity**	**Exposures**	**Time period for Exposures**
1. Washing of carrots	Start time: End time:	a) 'Silver' (wire) sponge used : Yes ( ) No ( ) b) Water used for washing dirty: Yes ( ) No ( ) c) Number of times water used for washing changed: ☐ d) Where wash water was disposed off: on bare ground ( ) drain ( ) Other (specify).............	e) Time period water used unchanged: ☐
2. Washing of Lettuce	Start time: End time:	a) Water used for washing dirty: Yes ( ) No ( ) b) Number of times water used for washing changed: ☐ f) Where wash water was disposed off: on bare ground ( ) drain ( ) Other (specify).............	c) Time period water used unchanged: ☐
3. Cutting/removal of waste parts of cabbage	Start time: End time:	a) Cabbage in contact with bare ground: Yes ( ) No ( ) b) Where cabbage displayed/stored after cleaning:	c) Time period cabbage in contact with bare ground:

		bare ground ( ) table top ( ) basket ( ) Other (specify)..........	
4. Cutting/removal of waste parts of spring onion	Start time: End time:	a) Spring onion in contact with bare ground: Yes ( ) No ( ) b) Where spring onion displayed/stored after cleaning: bare ground ( ) table top ( ) basket ( ) Other (specify)..........	c) Time period spring onion in contact with bare ground:
5. Unpacking of produce from sacks/polythene bags	Start time: End time:	a) Where produce unpacked to: bare ground ( ) table top ( ) basket ( ) Other (specify)..........	b) Time period produce in contact with bare ground:
6. Sweeping at vending site	Start time: End time:	a) Rubbish collected immediately after sweeping: Yes/No b) Where rubbish was kept in: Basket ( ) Polythene bag ( ) taking to skip container outside/within market ( ) Other ( ) c) Is rubbish collected covered if it is kept at vending site? Yes ( ) No ( )	d) Time period rubbish kept at vending site: e) Time period rubbish kept at vending site uncovered:
7. Packing/displaying of produce for sale	Start time: End time:	a) Where produce unpacked to: bare ground ( ) table top ( ) basket ( ) Other (specify).......... b) Flies hovering on produce: Yes ( ) No ( )	
8. Sprinkling of water unto	Start time:	a) Type of water used: piped water ( ) well water (	

produce	End time:	) Sachet water ( ) Other (specify)............ b) How water was sprinkled: hand ( ) Sachet water ( ) use of foam ( ) Other (specify)..........	
9. Eating	Start time: End time:	a) Vendor washed hands: Yes ( ) No ( ) b) Vendor washed hands with water and soap: Yes ( ) No ( ) c) Type of water used to wash hands: Piped water ( ) well water ( ) Sachet water ( ) Other (specify)............	
10. Vendor Idle (Indicate activities)	Start time: End time:		

Enumerator ID: ***Location ID:*** ***Date:***

Annex 4c: Observation guide for street food vendors

Observation Guide – Behaviour Record (Street Food Vendors)
For each activity, record the time for performing the activity and also complete the exposure parameters. Observe the Vendor from 6 – 9 pm.

Activity/Item	**Time period for activity**	**Exposures**	**Time period for Exposures**
1. Preparation of Salad	Start time: End time:	a) Vendor washed hands before handling salad: Yes ( ) No ( ) b) Source of water used to wash vegetables (ask vendor if not sure of the source) :Piped water ( ) Sachet water ( ) Well water ( ) Other (specify)....................... c) How vegetables were washed: Water only ( ) Salt water ( ) Vinegar ( ) Other (Specify)..............	
2. Storage of salad		a) Type of receptacle salad is kept in before serving to customers (Specify) ☐ b) Is salad covered in receptacle? Yes ( ) No ( )	c) Time period salad was left uncovered: ☐
3. Serving of rice with salad	Start time: End time:	a) Was salad covered before serving was done? Yes ( ) No ( ) b) How salad is served to customers: Hand ( ) Spoon/Ladle ( ) Other (specify)....................	d) Time period salad was left uncovered: ☐

		c) How chicken/meat/fish is served to customers: Hand ( ) Spoon/Ladle ( ) Other (Specify).................	
4. Washing of Plates	Start time: End time:	a) Source of water used to wash plates: Piped water ( ) Sachet water ( ) Well water ( ) Other (specify)...................... b) Number of times water used for washing plates changed: ☐ c) Where wash water was disposed off: On bare ground ( ) drain ( ) Other (specify).............	d) Time period before water used to washed plates was changed: ☐
5. Eating	Start time: End time:	a) Vendor washed hands: Yes ( ) No ( ) b) Vendor washed hands with: Water only ( ) Water and soap ( ) Other (specify)................. c) Type of water used to wash hands: Piped water ( ) well water ( ) Sachet water ( ) Other (specify)............	
6. Vendor Idle (Indicate activities)	Start time: End time:		
7. Number of Customers	Tally the number of customers within the		

	observation period		
8. Environmental Conditions at vending site		a) How refuse is managed/kept at vending site: In a covered receptacle ( ) In uncovered receptacle ( ) Left on the vending ground ( ) Other (Specify)................................... b) Vending site concreted: Yes ( ) No ( ) c) Floor of vending site swept/kept clean: Yes ( ) No ( ) d) Vending site within 3m of open drain: Yes ( ) No ( ) e) Flies at vending site: Yes ( ) No ( )	

Enumerator ID: ***Location ID:*** ***Date:***